THE EFFECTS OF DIABETES MELLITUS ON THE RELATIONSHIP BETWEEN MYOCARDIAL ADENOSINE PRODUCTION AND CORONARY VASCULAR RESISTANCE

1987

WARNER

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Relationship Between Myocardial Adenosine
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ABSTRACT

Title of Dissertation: THE EFFECTS OF DIABETES MELLITUS ON THE RELATIONSHIP
BETWEEN MYOCARDIAL ADENOSINE PRODUCTION AND CORONARY VASCULAR RESISTANCE

Eve Lynn Warner, Doctor of Philosophy, 1987

Dissertation directed by: Jack E. McKenzie, Ph.D.
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Adenosine, a product of adenosine triphosphate metabolism, has been
proposed as a mediator of coronary vascular resistance (CVR) in the normal
heart. This study was designed to determine if the relationship between
adenosine production and CVR is altered in diabetes mellitus, a metabolic
disorder characterized by a decrease in insulin function and associated with
serious cardiac complications. The relationship between myocardial adenosine
production and CVR was studied in normal and chemically - induced diabetic rats
and dogs. There was a significant negative correlation between myocardial
adenosine content and CVR in the normal rats, but not in the diabetic rats.
Diabetic rats exhibited decreased coronary blood flow (CBF) and impaired
ventricular pressure development and contractility as compared to controls.
Myocardial adenosine content was elevated in the diabetic rats without an
associated change in CVR. The effects of exercise conditioning on the
relationship between myocardial adenosine content and CVR were also tested
because exercise training has been shown by others to increase insulin binding
and glucose uptake in blood cells and skeletal muscle, respectively, in both
normal and diabetic individuals. Following an eight - week running program,
the correlation between myocardial adenosine content and CVR was restored to
normal in the diabetic rats. Studies were conducted in dogs for the purpose of

studying adenosine release, or the product of CBF and coronary sinus - arterial adenosine concentrations. Under basal conditions, diabetic dogs exhibited decreased CVR and increased coronary blood flow, cardiac work and myocardial oxygen consumption as compared to normal animals. Interestingly, basal adenosine release was not different between the two groups and a significant negative correlation between adenosine release and coronary vasodilatation during intravenous norepinephrine infusion was evident in normal dogs only. Intravenous norepinephrine caused a two-fold increase in cardiac work and coronary blood flow in both normal and diabetic dogs. The finding that intracoronary injections of adenosine elicited similar vasodilatory responses in normal and diabetic dogs suggests that diabetes mellitus attenuates the stimulated release of adenosine. Since insulin administration in this study increased adenosine sensitivity of the coronary vasculature only in normal dogs it further appears that metabolic control of CVR is greatly diminished in diabetes mellitus. Overall, these data confirm that an inverse relationship exists between myocardial adenosine production and CVR in normal animals, and indicate that adenosine may not play a prominent role as a mediator of coronary blood flow in diabetic animals.

THE EFFECTS OF DIABETES MELLITUS

ON THE RELATIONSHIP BETWEEN MYOCARDIAL

ADENOSINE PRODUCTION AND CORONARY VASCULAR RESISTANCE

by

EVE LYNN WARNER

Thesis submitted to the Faculty of the Department of Physiology Graduate

Program of the Uniformed Services University of the

Health Sciences in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy 1987.

This work is dedicated to the memories of Moe, Larry, Curly, Bowie, Tiffany, Speckles, Winey, Springer, Ubu, Scruffy and Wimpy, who found themselves here due to neglect or a roving curiosity and left with a purpose, fair treatment and love. As I look at each data point on each graph I will remember the trusting eyes and wagging tails of those who emerged from frightened strangers to faithful friends.

ACKNOWLEDGEMENTS

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Animal Groups

Rat Study

SC	Sedentary Control	RC	Running Control
SD	Sedentary Diabetic	RD	Running Diabetic

Dog Studies

NC	Normal Control	NNE	Normal/Norepinephrine
DC	Diabetic Control	DNE	Diabetic/Norepinephrine
PC	Prazosin/Normal/Control	PNE	Prazosin/Normal/Norepinephrine
DPC	Diabetic/Prazosin/Control	DPNE	Diabetic/Prazosin/Norepinephrine

Text

ADOR	adenosine release/uptake
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
CBF	coronary blood flow
CO	cardiac output
CrP	creatine phosphate
CS	coronary sinus
CVR	coronary vascular resistance
dP/dt	change in left ventricular pressure/change in time
HR	heart rate
L/P	lactate/pyruvate ratio
LVP	left ventricular pressure
MABP	mean arterial blood pressure
MVO_2	myocardial oxygen consumption
NE	norepinephrine
P	prazosin
PRP	pressure rate product

I   BACKGROUND

A. Introduction

The study of blood flow regulation in the heart has predominantly
been carried out in healthy animals. However, in disease states resulting
in cardiac dysfunction, regulatory mechanisms may be altered either as
homeostatic responses or as part of the etiology of the disease.
Therefore in order to further understand determinants of cardiac function,
it is valuable to study coronary blood flow regulation in disease as well
as in health.

Diabetes mellitus is a metabolic disorder associated with serious
secondary complications, including those affecting the cardiovascular
system. Cardiovascular disease is the leading cause of death in the
diabetic population and diabetics are twice as prone to suffering a heart
related illness compared to non - diabetics (Chobanian et al., 1982;
Scott, 1975). This study will examine coronary blood flow regulation in
diabetes with particular interest in the role of adenosine, a cardiac
metabolite proposed as a physiologic mediator of coronary blood flow.

B. Coronary blood flow regulation

Blood flow through the coronary vascular bed is controlled by the
aortic perfusion pressure, or mean arterial blood pressure, and by
coronary vascular resistance. Since mean arterial pressure is usually
held constant, changes in flow are usually dependent on changes in
resistance (Scott et al., 1980). Vascular resistance is defined as the

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ratio of pressure drop along a vessel to blood flow, or how much of a pressure difference it takes to cause a certain flow (Heller and Mohrman, 1981). In the heart the resistive pressure drop is measured as the mean systemic arterial pressure minus right atrial pressure, and coronary resistance is derived by dividing the resistive pressure drop by coronary blood flow.

There are several determinants of fluid flow through a hollow tube that can be applied to flow through blood vessels. As shown in the following equation, known as Poiselle's equation, vessel radius plays a prominent role in regulating resistance and therefore blood flow:

$$Q = \frac{\Delta P \ \pi r^4}{8 \ l \ n}$$

where:

r = vessel radius
l = tube length
n = fluid viscosity

Q = flow
ΔP = pressure difference

Since vessel length and blood viscosity are usually unvarying, changes in resistance are usually due to changes in vessel radius. According to Poiselle's equation, small changes in vessel radius cause large changes in blood flow. All blood vessels except capillaries contain vascular smooth muscle and are capable of adjusting vessel radius by calcium - dependent contraction or relaxation. This vasomotion is greatest in arterioles, which contain relatively more smooth muscle than arteries or veins and

provide the largest resistive pressure drop along the vascular tree by their ability to change vessel radius (Berne and Levy, 1977). It is believed that arteriolar diameter is adjusted by a combination of metabolic and neural influences and the regulation of coronary vascular resistance is an area of active research.

1. Cardiac metabolism

The regulation of blood flow through an organ by metabolism holds that changes in flow must correlate positively with changes in metabolism (Berne, 1964). The energy available to actin and myosin for crossbridge formation in cardiac muscle is derived from the supply of high energy phosphates, adenosine triphosphate (ATP) and creatine phosphate (CrP). The versatility of the heart in utilizing a range of substrates for ATP production is recognized by its ability to use glucose, fatty acids, lactate, pyruvate, and ketones at varying proportions according to circulating substrate concentrations, hormonal status, blood gas concentrations, and work - dependent energy demand of the heart (Montini et al., 1981). ATP is derived from these substrates through oxidative and glycolytic means, but the heart functions under predominantly oxidative metabolism. Under anaerobic conditions cardiac energy production cannot be sustained by glycolysis alone, and ischemic damage will occur if anoxia is prolonged.

Under normal circumstances, the heart is an aerobic organ which utilizes oxygen at an average rate of 9 ml oxygen per minute per 100g. In the basal state, 60% of the oxygen consumed by the heart is derived by the oxidation of fatty acids, 25% of the oxygen consumed is due to glucose

metabolism, and 11% is due to lactate metabolism (Opie, 1969). In healthy individuals, fourteen percent of the oxygen consumed in oxidative phosphorylation is used for energy involved in external work (Berne and Levy, 1977). The remainder of the oxygen consumed is used for non - contractile cellular processes or is liberated as heat. Mitochondria comprise nearly one - half of the myocyte content, allowing for increases in oxygen consumption up to four times the basal rate in conditions such as during exercise. When oxygen is not available to accept hydrogen ions from the electron transport chain, anaerobic means are used for providing ATP to the contractile elements. This causes a shift from the domination of fatty acid metabolism to anaerobic glucose metabolism, with the accumulation of lactate. In diabetes, where glucose transport and utilization are depressed, glycolytic protection from ischemia may be impaired, which could account for the lower incidence of recovery after myocardial infarction in diabetic patients (Opie *et al.*, 1979).

The heart extracts 75% of all the oxygen carried by the blood in a single pass through the coronary circulation. When cardiac work increases, cardiac metabolism must increase to provide more ATP to the contractile elements. In order to meet the increased myocardial oxygen demand, either oxygen extraction or coronary blood flow must increase. Since oxygen extraction is already near maximal in the basal state, increases in blood flow match oxygen delivery to oxygen demand. Because cardiac metabolism and oxygen consumption are closely linked, myocardial oxygen consumption has been used as an index of cardiac metabolism, and has been shown to correlate with increases in cardiac work (Feigl, 1983).

Cardiac work can increase in several ways to produce increases in oxygen consumption. First myocardial tension development, represented by ventricular pressure, has a strong influence on cardiac metabolism. Myocardial tension development can be increased by either increased ventricular filling, known as preload, or by increasing aortic pressure, known as afterload. Heart rate also plays a role in determining cardiac oxygen consumption (Braunwald, 1971). As heart rate increases, metabolism must increase to enable more cycles of tension development per unit time. A third consideration in the determination of myocardial oxygen consumption is the contractile state of the heart. Increases in contractility are defined as increases in the force of contraction independent from changes in preload. Contractility, which can be represented by maximum velocity of pressure development (dP/dt), can be influenced by sympathetic stimulation or $beta_1$ - adrenergic agonists such as epinephrine, norepinephrine, and isoproterenol.

The close matching of coronary blood flow to myocardial oxygen consumption, and therefore cardiac metabolism, has led to the examination of the metabolic regulation of coronary blood flow. According to the theory of metabolic blood flow regulation, the link between metabolism and blood flow involves either a substrate or metabolic by - product, which acts on vascular smooth muscle to alter vascular resistance. Accordingly, the agent enters the interstitial space from the tissue cells at a rate which is proportional to tissue metabolism and leaves the interstitium at a rate which is proportional to blood flow.

During increases in cardiac work it is believed that increased metabolism causes the release and build - up of a vasoactive metabolite or metabolites in the interstitial space. These agents in turn act on vascular smooth muscle to cause an arteriolar relaxation, a decrease in vascular resistance and an increase in coronary blood flow. The substance is continuously released while being washed out, attaining a balance between oxygen supply and demand. This process, in the case of exercising muscle, is known as active hyperemia, and occurs when the balance of oxygen supply to demand is altered specifically by increases in oxygen demand (Berne, 1974). The relationship between myocardial oxygen consumption and coronary blood flow due to active hyperemia at different levels of cardiac work is illustrated in Figure 1.

In another situation, blood flow is increased after a period of oxygen lack in order to meet basal metabolic demands. For example, after an arterial occlusion blood flow exceeds the basal level for a period of time proportional to the duration of the occlusion. This is known as reactive hyperemia and is presumably due to the build-up of vasodilator metabolites during the period of occlusion (Olsson, 1975). A decrease in oxygen supply due to hypoxia is also associated with an increase in blood flow which is attributed to the release of vasoactive metabolites.

Metabolic control of vascular resistance is thought to play a role in coronary autoregulation. Autoregulation is the ability of a vascular bed to maintain a constant flow in the face of changing perfusion pressures. Although mean arterial blood pressure stays relatively constant under normal conditions, there are variations in blood pressure

Figure 1. Relationship between coronary blood flow and myocardial oxygen consumption. Solid circles denote control conditions, partially filled circles denote hemorrhage or intravenous administration of epinephrine, and triangles denote 2,4 - dinitrophenol administration. (From Rubio et al., 1974).
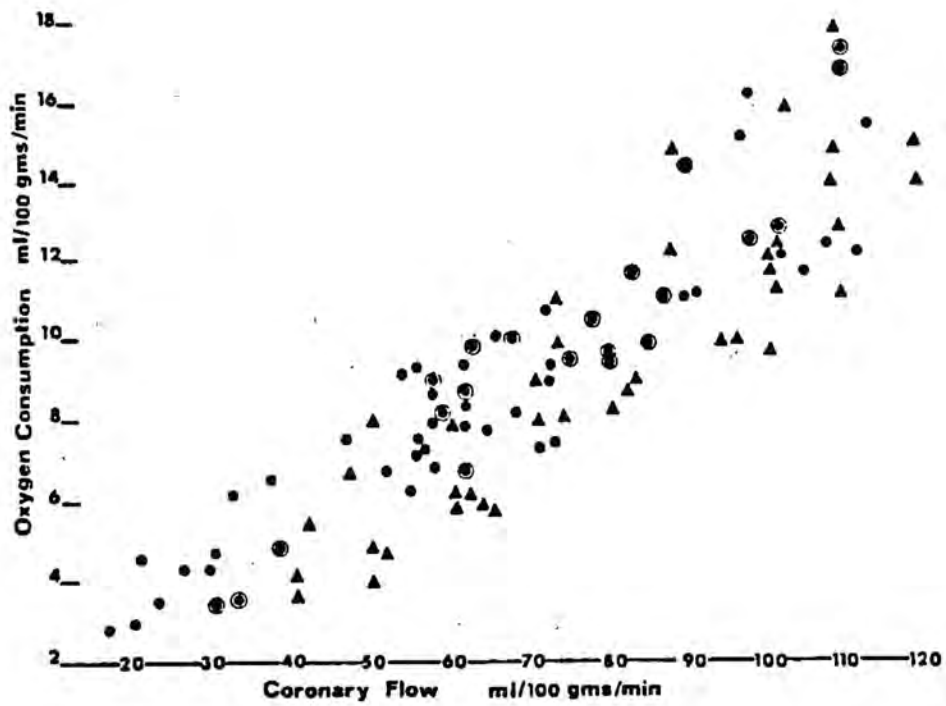
at different times. When arterial pressure is increased, for example, coronary input pressure increases, causing an increase in blood flow. Under these conditions the vasoactive metabolites present in the vascular bed are thought to be washed out, resulting in an increase in vascular resistance and a decrease in coronary blood flow to the original level. On the other hand, a reduction in blood pressure is thought to decrease coronary blood flow and increase the concentration of vasoactive metabolites, thereby causing vasodilation and the reestablishment of the original flow.

Several substances known to be released from cardiac tissue and found in the cardiac interstitium are capable of producing vasodilation. It is probable that a combination of these vasoactive agents, and not merely one substance, is responsible for the metabolic regulation of coronary blood flow. Oxygen, carbon dioxide, pH, potassium, osmolarity and adenosine all are likely candidates for mediators of coronary vascular resistance.

a. Oxygen

The correlation between myocardial oxygen consumption and coronary blood flow, and the observation that coronary venous oxygen tension remains relatively constant with changes in myocardial metabolism, have led to the suggestion that oxygen is a possible mediator of coronary vascular resistance. In a study by Belloni and Sparks (1977), coronary blood flow was held constant during stepwise increases in heart rate in order to measure changes in coronary vascular resistance with decreases in coronary venous oxygen content due to increases in myocardial oxygen

consumption. Since decreases in coronary venous oxygen content preceded decreases in coronary vascular resistance, this study indicated that a mechanism involving oxygen consumption could influence coronary blood flow, but it did not prove that oxygen itself was the mediator.

It has been known for many years that hypoxia causes coronary vasodilation and that hyperoxia causes coronary vasoconstriction (Gremels and Starling, 1926; Eckenhoff et al., 1947). Several laboratories have demonstrated hypoxic vasodilation by perfusing hearts with hypoxic blood, and it seems that coronary vasodilation occurs when coronary venous oxygen content or tension fall below 5.5 ml/100ml or 18mmHg, respectively (Berne et al., 1957; Nakamura et al., 1969; Vance et al., 1971). In these studies arterial oxygen tension had to be less than 40 - 50 mmHg in order for coronary venous oxygen content to decrease. Since this threshold arterial oxygen content is not within physiological range, a direct effect of oxygen tension on coronary resistance vessels was not indicated. In contrast, no thresholds in arterial or coronary venous oxygen tensions were needed to produce vasodilatory responses to graded arterial hypoxia in dogs with adrenergic receptor blockade (Powers and Powell, 1973). In studying the importance of oxygen tension in reactive hyperemia, Olsson (1975) reperfused the coronary vasculature with hyperoxic blood after periods of occlusion and found that there were no decreases in reactive hyperemic responses with high oxygen concentration. This experiment supported the idea that a decrease in oxygen supply probably triggers the release of another substance that is directly responsible for the vasodilation.

Coronary vasoconstriction has been observed with breathing 100%

oxygen and with exposure to hypobaric oxygen tensions (Ganz et al., 1972;

Ledingham et al., 1971). Since autonomic reflexes cause decreases in

myocardial oxygen consumption with hyperoxia, it is difficult to attribute

the hyperoxic vasoconstriction to oxygen alone. Lammerant and coworkers

demonstrated a 10% reduction in coronary blood flow with autonomic

blockade, whereas a 20% reduction in coronary blood flow was found without

autonomic blockade (1969). These results are in favor of oxygen playing a

role in the control of vascular tone but again do not show that oxygen is

the vasoactive agent.

b. Carbon dioxide

Carbon dioxide (CO_2), an end product of metabolism, is released

from cells in proportion to metabolic rate. As shown by Markwalder and

Starling in 1913, hypercapnia elicits vasodilation in the coronary

circulation. However, as suggested by Rooke and Sparks (1980), the

effects of CO_2 seem to be too small to account for the magnitude of

hyperemia associated with sympathetically - mediated increases in oxygen

consumption. The affects of CO_2 may be due to consequent decreases in pH

(Berne, 1974), and adenosine sensitivity has been shown to be enhanced by

decreases in pH (Merrill et al., 1978). Therefore, CO_2's role in

regulating coronary vascular resistance could be through an indirect

potentiation of adenosine's vasodilatory capabilities.

c. Potassium

Potassium ion (K$^+$) is released from skeletal muscle during exercise and is considered to be a possible mediator of skeletal muscle blood flow. Potassium may also play a role as a physiological coronary vasodilator although it is only capable of causing vasodilation at low concentrations (1 - 10 mM), whereas it causes vasoconstriction at high concentrations (> 15 mM) (Feigl, 1983). Increased myocardial K$^+$ release has been demonstrated when cardiac work was increased by increasing preload, afterload or heart rate (Cingolani and Dutrey, 1964; Sarnoff et al., 1966). However, the changes in coronary venous K$^+$ concentration were only transient compared to the length of the vasodilation. Increases in coronary venous K$^+$ concentration were also observed during reactive hyperemia after a 30 second occlusion (Scott and Radawski, 1971). In contrast, increases in coronary blood flow due to sympathetic stimulation and catecholamine infusion were not accompanied by increased K$^+$ release from the heart (Driscol and Berne, 1957; Gerlings et al., 1969; Sarnoff et al., 1966).

d. Osmolarity

Perfusion studies of the coronary circulation have shown decreases in vascular resistance with hyperosmotic solutions of sodium chloride, dextrose, or urea, and increased vascular resistance with hypoosmotic blood (Brace et al., 1975; Gazitua et al., 1971). However, increases in coronary venous osmolarity have not been demonstrated with sympathetic stimulation or reactive hyperemia, and a role, therefore, of osmolarity in the local metabolic control of coronary blood flow has not

been substantiated (Scott and Radawski, 1971).

e. Adenosine

Adenosine, a breakdown product of ATP, is considered to play a prominent role in the mediation of coronary vascular resistance. In 1963, Berne hypothesized that adenosine traversed the myocyte cell membrane to cause relaxation of coronary vascular smooth muscle. Since his hypothesis, research directed towards testing the "Adenosine Hypothesis" has evaluated how adequately adenosine fulfills the following criteria for a metabolic regulator of coronary blood flow: 1) The substance must be a potent dilator of coronary resistance vessels. 2) There must be an endogenous source of the mediator. 3) The substance should have access to the arterioles and be present under basal physiological conditions. 4) The concentration reached in the interstitial fluid must be capable of eliciting vasodilation, and there should be a close relationship between interstitial fluid concentration of the substance and coronary blood flow. 5) The time course of increased oxygen demand should parallel the increment in coronary blood flow. 6) The physiological effect at different concentrations of the endogenous mediator should be mimicked by exogenous administration of the substance. 7) Agents that potentiate or attenuate the action of administered mediator should elicit a similar effect on endogenously liberated mediator. 8) A direct cause - and - effect relationship should be established under all physiological and pathological conditions between changes in coronary blood flow and the substance's release (Berne, 1980). Although a direct cause - and - effect relationship between adenosine release and coronary blood flow has not

been demonstrated, adenosine fulfills most of the criteria set for a metabolic regulator of coronary blood flow.

Adenosine is a potent coronary vasodilator, as shown by infusion studies in isolated perfused hearts, intact blood perfused hearts, and isolated coronary arteries (Schrader et al., 1977; Herlihy et al., 1976). Infused adenosine may act on cell membrane receptors on vascular smooth muscle located either on the lumenal side or on the basal side of the vascular smooth muscle after diffusion through the cell. It is speculated the adenosine receptor types may differ depending on location on the vessel membrane (Olsson et al., 1976a). Adenosine is thought to cause smooth muscle relaxation through a decrease in inward calcium flux at concentrations ranging from 10^{-7} to 10^{-5} M (Fenton et al., 1982). This response may be mediated via an increase in cyclic adenosine monophosphate (cAMP), whereas adenosine mediated decreases in calcium influx in cardiac muscle are probably caused by decreases in cAMP through another adenosine receptor type (Londos et al., 1983).

The scheme for the formation and metabolism of endogenous adenosine is shown in Figure 2. Adenosine can be formed in the heart from adenosine monophosphate (AMP) by the enzyme 5'-nucleotidase, or from S-adenosylhomocysteine (SAH) by the enzyme SAH hydrolase (Schrader, 1983). Conversion of AMP to adenosine by 5'-nucleotidase can occur both in the cytoplasm and at the cell membrane. Originally it was thought that the membrane bound enzyme is responsible for the formation and immediate release of adenosine when adenosine formation is stimulated (Berne, 1980). However, recent evidence indicates that most cardiac adenosine is probably

Figure 2. Myocardial adenosine formation and degradation. Adenosine formed in the myocyte by cytoplasmic 5'-nucleotidase is transported across the cell membrane, stored as S - adenosyl homocysteine (SAH), or degraded by adenosine deaminase (ADA) to inosine. Inosine is degraded to hypoxanthine by nucleoside phosphorylase (NP) intracellularly, in the interstitium, target cell, or blood. Adenosine acts via extracellular receptors to stimulate or inhibit the formation of cAMP.
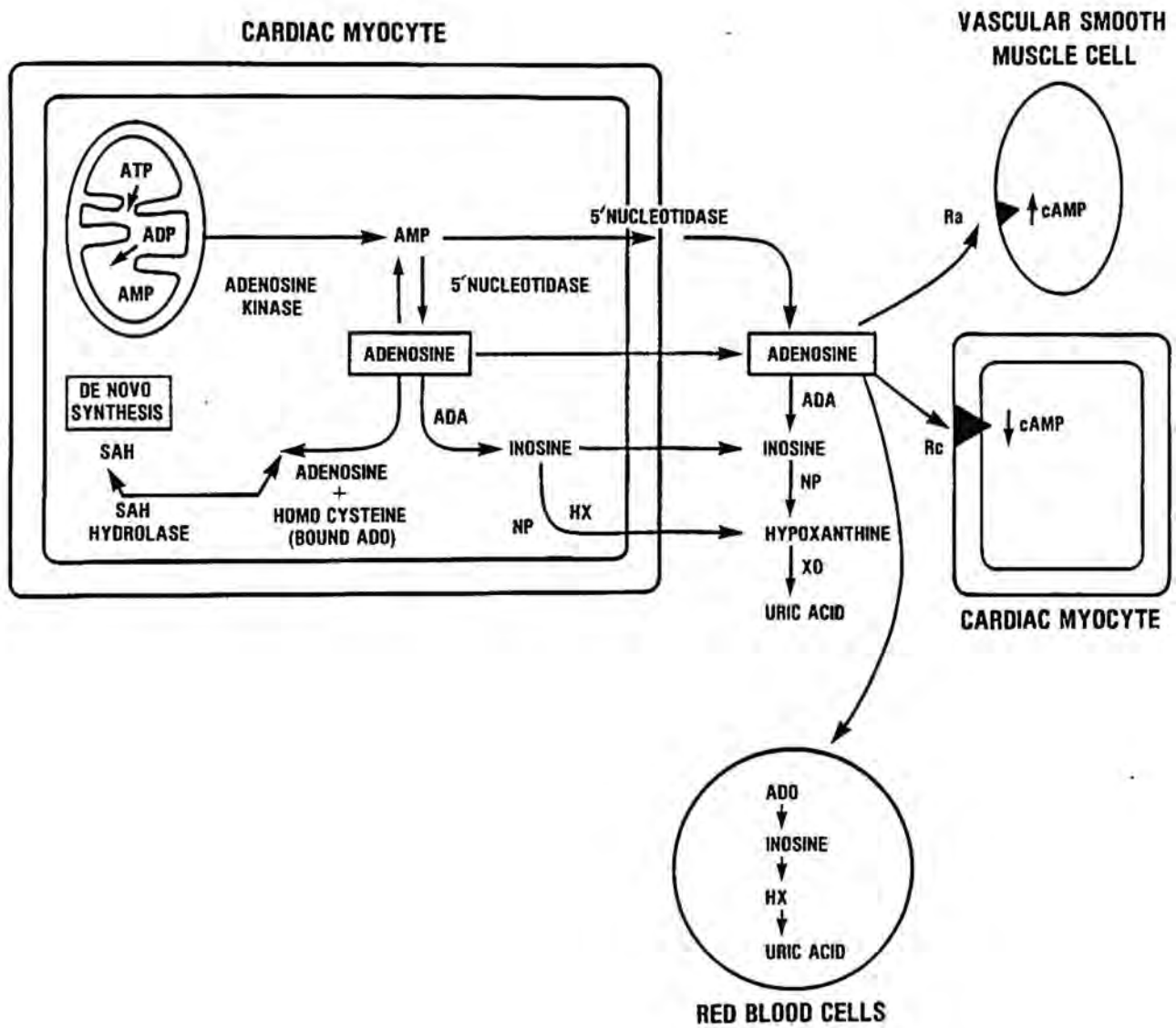
formed intracellularly and is subsequently released into the extracellular

fluid by passive and facilitated diffusion (Belloni et al., 1985). The

formation of adenosine by SAH hydrolase occurs in the cytoplasm, and SAH

and adenosine bound to SAH hydrolase are protected from enzymatic

degradation. The release of adenosine from SAH into the interstitium has

not yet been observed, so it is assumed that the major fraction of the

adenosine produced and released under a variety of challenges to the heart

is derived from AMP (Schrader and Gerlach, 1976).

In order to understand the control of adenosine production,

regulation of 5'-nucleotidase activity has been studied. Under basal

conditions, 5'-nucleotidase is inhibited by adenosine triphosphate (ATP),

adenosine diphosphate (ADP), and creatine phosphate (CrP) (Baer et al.,

1966). One theory is that when cellular levels of CrP decline, as would

occur when the rate of dephosphorylation of high - energy phosphates

exceeds the rate of rephosphorylation, the inhibition of 5'-nucleotidase

is removed, and adenosine production is increased. Magnesium ion, which

is liberated during the breakdown of ATP, also releases the inhibition of

5'-nucleotidase (Sullivan and Alpers, 1971). A cellular control system

for modulating the activity of SAH hydrolase has yet to be established.

Once adenosine is released into the interstitial fluid it can act

on extracellular receptors on vascular smooth muscle to decrease vascular

resistance (Olsson et al., 1976b, Schrader et al., 1978). It has also

been proposed that adenosine causes vasodilation via the presynaptic

inhibition of norepinephrine release from adrenergic nerve terminals,

thereby decreasing alpha - adrenergic vasoconstrictor tone (Hedqvist and

Fredholm, 1979). Other cardiac effects of adenosine that might occur

during conditions where adenosine release is enhanced include reductions

in sinoatrial node activity, atrioventricular node conduction, and atrial

and ventricular contractility (Collis, 1983; Heller and Olsson, 1985).

Free extracellular adenosine is subject to degradation in the

lymph and plasma by the enzyme, adenosine deaminase. Adenosine also

enters endothelial cells and red blood cells by facilitated diffusion,

followed by enzymatic degradation to inosine (Schrader et al., 1972). In

addition, adenosine is taken up by myocardial cells and rephosphorylated

to AMP.

Adenosine has been measured in myocardial tissue and in arterial

blood during basal conditions and during hypoxia and reactive hyperemia.

Although interstitial adenosine concentrations cannot yet be measured with

present technology, assumed correlates of interstitial adenosine, such as

tissue, coronary sinus, and cardiac lymph adenosine concentrations, have

served as estimates of changes in interstitial values. It has been shown

in our laboratory that myocardial tissue adenosine content, cardiac lymph

and coronary sinus adenosine concentrations correlate closely enough to

allow one to assume that changes in these values probably reflect changes

in adenosine release into the interstitium (McKenzie et al, 1984). Normal

resting myocardial adenosine concentrations in in - vivo dogs and rats

range between 0.20 - 0.85 nmoles/g wet weight and 3.6 to 8 nmoles/g wet

weight, respectively (Berne et al., 1971; Degenring et al., 1975; Foley et

al., 1979; McKenzie et al., 1980; Olsson, 1970). The concentration of

adenosine in canine arterial blood under resting conditions is

approximately 0.3nM (McKenzie et *al.*, 1984).

In a study by Rubio et *al.* (1974), adenosine measured in coronary effluent was shown to increase with hypoxia to concentrations adequate to cause vasodilation, and adenosine release correlated with increases in coronary blood flow. In 1980, Saito and coworkers measured tissue adenosine during active hyperemia caused by atrial pacing and aortic constriction. McKenzie et *al.* recovered coronary sinus and tissue adenosine during increases in cardiac work caused by aortic constriction, isoproterenol infusion, and acute exercise (1980, 1981, 1982, 1985). In both Saito's and McKenzie's laboratories, adenosine correlated positively with oxygen consumption and negatively with coronary vascular resistance. Manfredi and Sparks did not find changes in adenosine and coronary blood flow to correlate during atrial pacing, but did conclude that adenosine was involved in the hyperemia associated with norepinephrine infusion (1982).

By studying coronary reactive hyperemia, Rubio et *al.* (1969) and Olsson and coworkers (1978) found tissue adenosine contents to increase after coronary occlusions greater or equal to five seconds in duration. In Olsson's study, correlation curves between endogenous myocardial adenosine content and coronary vascular conductance (1/resistance) during reactive hyperemia were compared with equally matched adenosine contents attained by adenosine infusion and coronary flow responses to infused adenosine. Since the slope of the endogenous adenosine content vs coronary vascular conductance was twice as steep as the exogenous adenosine vs conductance curve, it was concluded that adenosine alone

could only account for half of the reactive hyperemic response. Several other groups have examined the effects of adenosine blockers on reactive hyperemia. Use of the methyl xanthine derivatives, theophylline and aminophylline, which have been shown to block vasodilation associated with adenosine infusions, have provided mixed results. In some cases, administration of these blockers attenuated the hyperemic response and in others, there was little or no effect (Feigl, 1983). In a recent study by Saito and coworkers (1985b), aminophylline was shown to diminish the reactive hyperemic response to 5, 10, 15, 20, and 30 - second coronary occlusions. In addition, a threshold dose of infused adenosine was found to enhance the reactive hyperemic response, and this enhancement was abolished by aminophylline. In another study by Saito et al., an intracoronary infusion of the adenosine degrading enzyme, adenosine deaminase, reduced reactive hyperemia flow by 30% (1981). Conversely, inhibition of endogenous adenosine deaminase with a non - vasoactive adenosine analog, was shown to enhance myocardial reactive hyperemia (Saito et al., 1985a).

2. Neural control of coronary blood flow

Coronary arteries are innervated by both parasympathetic and sympathetic fibers (Lever et al., 1965). The parasympathetic cholinergic neurons cause vasodilation in the dog coronary vasculature, and vasoconstriction in the domestic swine and in man (Feigl, 1969; Ginsburg, 1984; Ito et al., 1979). The physiological significance of coronary cholinergic innervation is presently unknown. However, in some clinical

settings and some experimental models, coronary vasospasm is linked with enhanced cholinergic activity.

Adrenergic receptors are found on coronary vascular smooth muscle and cardiac muscle. Alpha$_1$ and alpha$_2$ - adrenergic receptors cause vasoconstriction and beta$_2$ - adrenergic receptors cause vasodilation of coronary resistance vessels. Myocardial beta$_1$ receptors cause increases in heart rate and contractility, and myocardial alpha$_1$ receptors have been shown to cause an increase in cardiac contractility in some species, such as the lamb, (Downing et al., 1983; Vatner et al., 1980).

Alpha-adrenergic vasoconstriction has been shown to be dominant over beta-adrenergic dilation during sympathetic stimulation (Segal and McKenzie, 1986). Sympathetic stimulation of the stellate ganglion causes increased heart rate, ventricular pressure and coronary blood flow. However, when cardiac beta$_1$ receptors are blocked, which prevents the increase in cardiac work, direct effects of sympathetic stimulation on coronary vessels can be observed, since there is no stimulus for metabolic dilation. Feigl (1967) and Nayler and Carson (1973) found vasoconstriction associated with stellate ganglion stimulation and beta - blockade. In a later study Feigl showed that the vasoconstricton was strong enough to cause a decrease in coronary sinus oxygen content, representing an increase in oxygen extraction (1975). In addition, alpha - blockade abolished the increase in coronary vascular resistance and the decrease in coronary sinus oxygen content. Morhman and Feigl quantified

the influence of alpha - mediated vasoconstriction in norepinephrine -

stimulated hearts with and without alpha - blockade (1978). They observed

that the alpha constriction reduces the metabolically mediated increase in

coronary blood flow by 30%, causing an increase in oxygen extraction.

Using a more physiological model, Murray and Vatner (1979) and Heyndrickx

et al. (1982) found enhanced coronary blood flow in alpha - blocked

conscious exercising dogs. Although most investigators believe that

$alpha_1$-adrenergic receptors are responsible for the vasoconstriction

observed with sympathetic stimulation, Holtz and coworkers reported that

vasoconstriction is attenuated more strongly by $alpha_2$ antagonists than

$alpha_1$ antagonists (1977). However, in the cat, Segal and McKenzie (1986)

were able to block sympathetically - mediated coronary vasospasm with the

$alpha_1$ blocker, prazosin. These studies indicate a role for neural

control of coronary vascular resistance during sympathetic stimulation,

but there is a question as to whether or not there is neural tone at rest.

Holtz et al. (1977) reported resting vasoconstrictor tone in the dog, but

Chilian et al. (1981) were not able to demonstrate sympathetic tone.

Segal and McKenzie (1986) did not find a significant increase in resting

coronary blood flow in the cat with intracoronary prazosin administration,

but there was a regional change in coronary vascular resistance.

It can be concluded from the above studies that metabolic and

neural factors may balance each other, and perhaps interact, to control

coronary vascular resistance. If one factor is changed, the other might

compensate. In the conscious dog, for example, there is a sharp rise in

adenosine release with strenuous treadmill exercise. Since running is associated with sympathetic stimulation, the increase in adenosine is presumably due to the combination of an increase in myocardial metabolism secondary to increased cardiac output, and an increase in coronary vascular resistance, secondary to alpha - adrenergic vasoconstriction. If prazosin, an $alpha_1$ - adrenergic blocking agent, is administered to the coronary bed prior to the bout of exercise, adenosine release does not increase to the same extent, although coronary blood flow does increase equally (McKenzie et al., 1985). Thus without sympathetic vasoconstriction during exercise, blood flow can increase with a lower concentration of vasodilator metabolites.

In a diseased state such as diabetes, both metabolism and autonomic function are affected. It is possible, therefore, that there may be alterations in relationships between metabolic and neural factors in regulating cardiac function. The following section will address cardiac complications of diabetes and how changes associated with the disease may be related to alterations in coronary blood flow regulation.

C. Symptomology of Diabetes

Diabetes mellitus is a metabolic disorder of varied etiology characterized by hyperglycemia, polyuria, polydipsia, and glycosuria. Due to the different forms of the disease, diabetes has been classified into two major categories: insulin dependent (Type I or juvenile onset) and non - insulin dependent (Type II or adult onset). Type I diabetics

comprise 5 - 10% of the total diabetic population, are usually symptomatic before 30 years of age, are ketosis prone, and may show either autoimmune, viral or toxic destruction of the insulin producing pancreatic beta - cells, resulting in hypoinsulinemia. Type II diabetics comprise 85 - 90% of the diabetic population, are usually obese, are not ketosis prone, and usually develop symptoms secondary to a decrease in insulin receptor function (Lebovitz, 1984).

Since 1921, when Banting and Best isolated insulin and proved its application in lowering blood glucose, great progress has been made in extending the lives of diabetics. Present therapeutic techniques involve insulin or sulfonylurea therapy, controlled diet, and exercise. However, even with improved glycemic control, both types of diabetics are faced with the development of cardiac disease, hypertension, vascular degeneration, retinopathy, neuropathy, and nephropathy, as well as clotting disorders and reduced resistance to bacterial infection (Chobanian et al., 1982). It is the prevalence of these secondary complications that has led to the investigation of insulin's role in regulating cellular systems in addition to those involved in energy homeostasis.

D. Insulin and Diabetic Metabolism

Whether there is destruction of pancreatic tissue and hypoinsulinemia, or decreased insulin receptor function and hyperinsulinemia, the symptoms of diabetes mellitus are caused by a functional lack of insulin. Insulin is a 9000 dalton molecular weight protein hormone that is secreted by the beta cells of the pancreatic

islets of Langerhans in response to a carbohydrate and/or protein meal, hyperglycemia, or parasympathetic activation. The best known role of insulin involves the promotion of energy storage in liver, skeletal muscle and adipose tissue. As illustrated in Figure 3, insulin promotes glycogenesis, glycolysis, and protein synthesis in liver, facilitates glucose and amino acid transport and protein synthesis in skeletal muscle, and increases glucose transport and protein synthesis while inhibiting lipolysis in adipose tissue (Berne and Levy, 1983). The hormone acts through membrane bound protein receptors that have tyrosine kinase activity. The insulin – receptor complex produces immediate effects such as membrane hyperpolarization, increased glucose transport, and release of chemical messengers before being internalized where it exerts its delayed metabolic effects, such as increased protein synthesis (Hollenberg, 1985). The insulin receptor does not act through adenylate cyclase (cAMP), as do the catabolic or counterregulatory hormones, glucagon, growth hormone, and catecholamines. These hormones act to release stores of glucose, amino acids, and fatty acids into the circulation by increasing glycogenolysis, lipolysis, and protein catabolism in liver, skeletal muscle, and fat.

In diabetes, the ratio of functional insulin to the catabolic hormones is decreased, and blood levels of energy substrates are increased. The primary symptoms of diabetes, at least partially, are a result of an imbalance of processes of energy mobilization such as glycogenolysis, proteolysis, and lipolysis, in relation to processes of energy storage such as substrate transport and the synthesis of glycogen, protein and fat. In addition to hyperglycemia, the state of catabolism in

Figure 3. Influence of insulin on glucose, amino acid, and fatty acid
transport and metabolism in muscle, liver and adipose. Solid arrows
denote stimulation and cross - hatched arrows denote inhibition.

MUSCLE   LIVER   ADIPOSE

GLYCOGEN

GLUCOSE   GLUC$-6-$Po$_4$ GLUCOSE

AMINO ACIDS

PYRUVATE CO$_2$

ffa

KETOACIDS

STIMULATES
INHIBITS } EFFECTS OF INSULIN
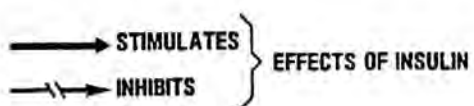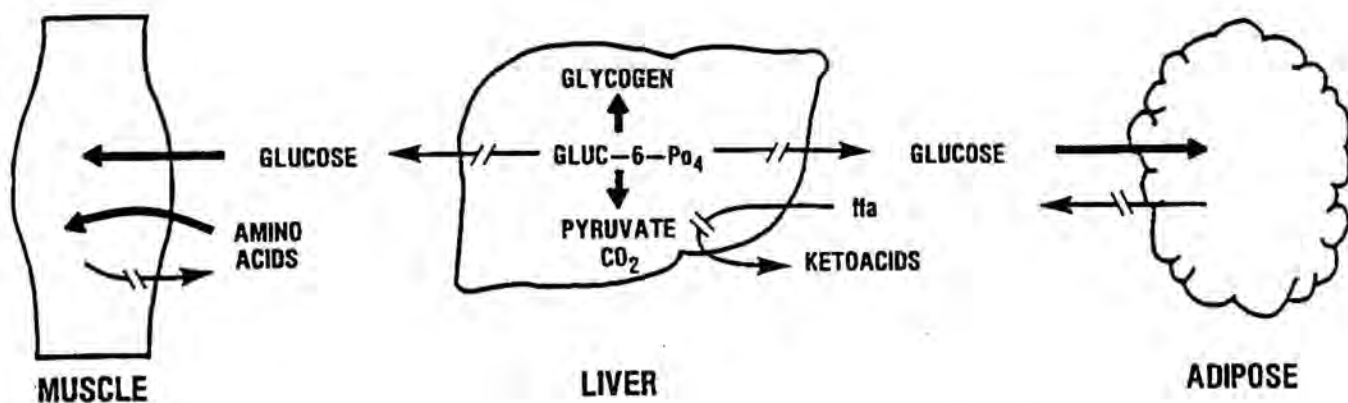
PLASMA
↓ GLUCOSE
↓ FFA
↓ KETOACIDS
↓ AMINO ACIDS

diabetes is associated with the formation of the ketone bodies, beta -

hydroxybutyrate and acetoacetate, which results in metabolic acidosis.

These molecules are synthesized in the liver from acetyl coenzyme A when

the rate of fatty acid oxidation exceeds the rate of entrance of acetyl

coenzyme A into the tricarboxylic acid cycle.

The diagnosis of diabetes mellitus is made when fasting venous

plasma glucose concentration is at least 140 mg/dl or greater than 200

mg/dl two hours after a 75 mg oral glucose tolerance test, or when plasma

glucose concentration is elevated along with the presence of polyuria,

polydipsia, ketonuria, and weight loss. The characteristic symptoms

usually occur with plasma glucose concentrations in excess of 300 mg/dl,

which exceeds the normal renal threshold for glucose reabsorption

(Lebovitz, 1984). In this case, glucose acts as an osmotic diuretic, and

if countermeasures are not taken the patient can become dehydrated and the

plasma becomes hyperosmotic. Ketones that aren't readily metabolized by

tissue are also spilled into the urine, and weight loss occurs due to a

generalized state of catabolism.

These symptoms can be minimized in the case of the juvenile

diabetic with insulin replacement and a diet that balances the insulin's

activity with nutrient intake. In the adult - onset diabetic oral

hypoglycemic agents, a low calorie diet and exercise are effective

therapeutic strategies. The regulation of blood glucose in each diabetic

patient must be an individualized program and there may be large

fluctuations in glucose levels due to periodic changes in insulin

sensitivity, with hypoglycemia and hyperinsulinemia posing as much of a

threat as hyperglycemia, dehydration, and ketosis.

Even the best monitoring efforts to stabilize glucose levels cannot assure constant normoglycemia. Many cell types, although not classically considered to be "insulin dependent," have been shown to be affected by poor glycemic control, and become damaged in long term diabetes. Cardiac muscle, vascular smooth muscle, and neural tissue, all involved in diabetic cardiovascular disease, have been shown to be sensitive to insulin and/or glucose levels (McMillan, 1975; Rubinstein *et al.*, 1984, Scott, 1975; and Young *et al.*, 1986). Alterations in metabolism of these tissues may be involved in the pathogenesis of the observed secondary complications.

E. Cardiac disease in diabetes

Metabolic alterations that affect several types of tissue found in the heart as well as circulating levels of energy substrates and hormones, accompany the primary symptoms of diabetes. An extensive amount of research has covered the development of the well documented characteristics of diabetic cardiac disease, which include atherosclerosis, microangiopathy, autonomic neuropathy, and cardiomyopathy. Recent evidence also suggests that there may be defects in the regulation of coronary blood flow.

Coronary artery disease is the most common cause of death in non - insulin dependent diabetes, but it is also found in juvenile diabetes (Knowles,1978). Adult diabetic patients are two to forty times more likely to develop coronary artery disease as compared to the general

population, depending on the presence of diabetes related and non – diabetes related coronary artery disease risk factors (Fuller, 1980). The high levels of circulating glucose, triglycerides, and cholesterol observed in diabetics are risk factors in the development of atherosclerosis. Diabetes related abnormalities including endothelial dysfunction, increased platelet adhesiveness, smooth muscle proliferation, increased collagen secretion, alterations in proteoglycans, and increased levels of low – density lipoproteins may contribute to the occlusive plaques (Cooper, 1984; Knowles, 1978). Both low and high levels of insulin and renin and high levels of growth hormone have also been implicated in atherosclerosis and diabetic macroangiopathy (Knowles, 1978).

Microangiopathy, or disease of small arterioles and capillaries, has been observed in cardiac and skeletal muscle, renal, retinal, and neural capillary beds (Fisher et al., 1981). Microangiopathy of the heart is not as prevalent as macroangiopathy, but may contribute to the decreased ventricular function seen in diabetics by limiting blood flow to discrete areas of the myocardium (Factor, 1984; McMillan,1975). The most common feature of diabetic small vessel disease is the presence of capillary basement membrane thickening, which can be observed in histological examinations, and may be accompanied by altered transport of metabolites across capillaries, microaneurysms, and areas of non perfusion (Factor et al., 1984 McMillan, 1975). Before basement membrane thickening can be observed, however, several early changes in vascular function occur. These changes include 1) increased local blood flow; 2)

progressive reversible dilation of small veins; 3) periodic arteriolar vasoconstriction; and 4) sclerosis of the wall of arterioles, small veins and capillaries (McMillan, 1975).

Possible contributing factors in the development of microangiopathy are numerous and involve the cellular elements of both the microcirculation and the blood. Decreased phagocytosis, accelerated cell aging and increased lysosomal enzymes could lead to endothelial cell damage through a decrease in protective mechanisms and an increase in processes of deterioration. Alterations in cell carbohydrate metabolism leading to an accumulation of sorbitol occur with hyperglycemia and may cause endothelial swelling through the osmotic properties of sorbitol (McMillan, 1975). Blood abnormalities include increased serum and blood viscosity and increased erythrocyte and platelet aggregation, which could increase the resistance of blood flow through the microvasculature. Hemoglobin glycosylation, in which either glucose or another sugar is attached to the hemoglobin molecule, is accelerated in diabetes and may lead to disturbances in oxygen transport. Glycosylated hemoglobin has a greater affinity for oxygen, so oxygen release at the tissues is inhibited. To overcome this problem, erythrocytes produce 2,3 - diphosphoglycerate, which decreases the affinity of hemoglobin for oxygen. Diabetic patients exhibiting microangiopathy have elevations in both glycosylated hemoglobin and 2,3 - diphosphoglycerate levels, but there is a greater increase in glycosylated hemoglobin than in 2,3 - diphosphoglycerate. Diabetic patients without signs of microangiopathy show equal elevations in both factors, which may signify that the increase

in oxygen affinity due to hemoglobin glycosylation is balanced by the decrease in oxygen affinity due to 2,3 - diphosphoglycerate. Growth hormone and glucagon, which are elevated in diabetics, are also implicated in promoting capillary fragility and increased serum viscosity, respectively (McMillan, 1975). Changes in patterns of the production of eicosanoids (prostaglandins, prostacyclin, thromboxane and leukotrienes) are observed in diabetes and may also contribute to the deterioration of the microcirculation through their effects on platelet function, vascular resistance and permeability (Rosen and Hohl, 1984; Rosen and Schror, 1980). Most of these contributing factors can be reduced with improved glycemic control, but glucose monitoring serves to slow down the processes more than preventing them.

Autonomic neuropathies in diabetes have been shown to involve damage to both sympathetic and parasympathetic nervous systems. The degeneration of sympathetic noradrenergic neurons to the heart causes variable rates of release of norepinephrine, and eventually leads to cardiac adrenergic receptor hypersensitivity to circulating catecholamines (Hoeldtke and Cilmi, 1984). This hypersensitivity is especially dangerous to diabetic patients because it renders them more prone to cardiac arrhythmias. For example, when an insulin injection is administered, hypoglycemia triggers the release of catecholamines from the adrenal medulla, and arrhythmias tend to develop at this time (LLoyd - Mostyn and Watkins, 1975). It has been suggested that there could be an increase in sympathetic tone in the diabetic rat heart (Paulsen and Light, 1981). A longitudinal study of the noradrenergic innervation of streptozotocin -

induced diabetic rat hearts showed increased levels of ventricular norepinephrine at one and two months of diabetes, and either normal or diminished levels of norepinephrine at eight months, depending on the age of the rats at induction of diabetes (Felten et al., 1982). Factor and Sonnenblick suggested that alpha mediated vasospasm could contribute to the alterations in blood flow regulation of the diabetic microcirculation (1982). Hart et al. found a decrease in norepinephrine content but an increased norepinephrine release from streptozotocin-induced diabetic rat caudal arteries (1985).

Parasympathetic neuropathy in diabetes is characterized by both afferent and efferent degeneration of the vagus nerve. When afferent cardiac nerves are destroyed, patients are unaware of ischemic episodes and may suffer from a myocardial infarction without the warning of pain (Cooper, 1984). Abnormally high heart rates and sinus arrhythmia, indicative of efferent vagal damage, are also prevalent in diabetic patients. Reduced cholinergic innervation, decreased axonal transport of acetylcholinesterase, and increased sensitivity to acetylcholine have been reported in experimentally diabetic animals, and could lead to an increased susceptibility to coronary vasospasm in species such as man, that exhibit coronary vasoconstriction in resonse to acetylcholine. Total autonomic denervation, in which neither sympathetic nor parasympathetic nerves are functioning, has been observed in some diabetic patients (LLoyd - Mostyn and Watkins, 1975). In these patients the baroreceptor reflex is abolished, leading to a fixed heart rate, orthostatic hypotension, and tendencies for cardiac arrhythmias.

The causes of autonomic neuropathy are probably a combination of microvascular changes which limit substrates and oxygen to the neural tissue, and metabolic alterations which affect energy production and axonal transport. In addition, sorbitol accumulation and subsequent axonal swelling are seen in diabetic autonomic neurons and may contribute to axonal dysfunction.(McMillan, 1975).

The prevalence of congestive heart failure in insulin - dependent diabetics has led to the examination of myocardial dysfunction in diabetes. The symptoms of cardiomyopathy, or disease of the cardiac muscle, are manifested by cardiac dilatation, hypertrophy, interstitial fibrosis, and focal necrosis (Badeer and Zoneraich, 1978). This aspect of diabetic cardiac disease has been examined in a variety of experimental animal models of diabetes. Isolated perfused hearts from alloxan - and streptozotocin - induced diabetic rats exhibited decreased ventricular pressure development at high filling pressures and preparations of isolated papillary muscle from alloxan - diabetic rabbits demonstrated delayed rates of shortening and relaxation when compared to normal preparations (Fein et al., 1985; Vadlamudi and McNeill, 1980; Vadlamudi et al., 1981). These observations were made before the development of anatomical changes that could lead to reductions in cardiac perfusion, but were within the possible time period of early changes in microvascular function and were reversible upon chronic insulin replacement. Abnormalities in cardiac contractility were also observed in an open-chest diabetic rat preparation in response to isoproterenol stimulation (Atkins et al, 1985).

The causal factors leading to a defect in cardiac function could be pinpointed in any of six areas involved in the cardiac contraction process. The sequence of events that lead to cardiac contraction are: 1) the provision of substrate for oxidative phosphorylation; 2) mitochondrial oxidative metabolism and the generation of ATP; 3) storage of high energy phosphates; 4) calcium release for excitation - contraction coupling; 5) contractile protein activation and 6) external mechanical work of pressure development (Chidsey, 1967). Experimental animal models of diabetes have provided information uncovering defects in several of these processes. First, although the heart is not considered to be dependent on insulin for its energy needs, it has been established that insulin deficiency causes reduced glucose transport and utilization and an increase in fatty acid metabolism in the hearts of diabetic dogs and rats (Opie, 1968). The alterations in substrate availability and the absence of insulin in regulating metabolic enzymes may be deleterious to mitochondrial generation of ATP. For instance, a reduction in glucose utilization could render the diabetic heart less protected against ischemic episodes, when glycolysis becomes an important process in providing energy for contraction. In addition, it has been suggested that in diabetes accelerated myocardial fatty acid oxidation increases levels of acyl coenzyme A, which may inhibit ATP - ADP translocase, thereby depressing mitochondrial ATP regeneration. Accordingly, myocardial levels of the high energy phosphates, ATP and creatine phosphate, have been shown to be depressed in diabetic animals (Opie et al., 1979).

The utilization of high energy phosphates for muscle contraction

depends upon the process of excitation - contraction coupling. During this process, a rise in intracellular free calcium triggered by cardiac muscle action potentials causes energy - dependent interaction of actin and myosin contractile protein filaments, which then results in muscle contraction. The establishment of the negative resting membrane potential by the sodium/potassium ATPase (Na^+/K^+ pump) and a proper ionic environment determines the excitability of cardiac cells. Once the cell membrane is depolarized, the permeability of the myocyte cell membrane to calcium increases, the sarcoplasmic reticulum releases calcium into the cytopolasm, and intracellular calcium concentration increases. This process is enhanced by the formation of cAMP in response to catecholamine stimulation. When the cytoplasmic calcium concentration is low, crossbridge interaction between actin and myosin is inhibited. When cytoplasmic calcium increases, calcium binds to a component of actin called troponin, and releases the inhibition of contractile ATPase so that crossbridges between actin and myosin form. The ATP dependent cycling of crossbridges is then transduced to perform work.

Reduced contractile function in diabetic animals could be attributed to several of the mechanisms mentioned above. First, membrane excitability could be altered in the diabetic myocyte. Insulin is known to stimulate Na^+/K^+ pump activity in normal cardiac muscle, whereas Na^+/K^+ pump activity has been shown to be reduced in diabetic rat hearts (Fein *et al.*, 1983; Ku and Sellers, 1982). Insulin replacement corrected the reductions in pump activity. Second, most of the defects in cardiac function observed in experimental animals, as mentioned above, have been

attributed to decreases in the ability of the sarcoplasmic reticulum to liberate calcium and to respond to adenylate cyclase (Atkins et al., 1985 Penpargkul et al., 1981). Third, decreased contractile ATPase activity observed in diabetic rat hearts could play a role in the development of diabetic cardiomyopathy (Malhotra et al.,1981) . Finally, an increase in ventricular stiffness attributed to increased collagen deposition found in the diabetic dog myocardium could limit ventricular filling and pressure development (Regan et al, 1981).

Although many investigators believe that decreased cardiac function is caused by changes in myocardial metabolism, there is growing interest in the possibility that coronary blood flow regulation may be impaired in diabetes (Factor et al., 1984). In 1955, Unger et al. found that diabetic patients had a tendency towards lower coronary blood flows as compared to normal subjects, but the differences were not statistically significant. Lee and Downing reported a significant decrease in coronary blood flow in diabetic newborn lambs, but studies in diabetic dogs revealed only a slight but not significant reduction in resting coronary blood flow when compared to normal animals (Koltai et al., 1984; Lee and Downing, 1979; Regan et al., 1981). In early stages of diabetes, such as during the first ten years of juvenile diabetes, blood flow has been reported to be increased in vascular beds such as in the kidney, retina, and heart, which eventually show microvascular and functional deterioration (Theusen et al., 1986). Renal hyperperfusion has also been observed within the first few weeks of diabetes in the chemically diabetic rat. These findings suggest that the relationship between metabolic

demand and blood flow or perhaps vascular reactivity to local metabolites may be altered in some vascular beds in diabetes. Since coronary blood flow must change to meet the changing metabolic demands of the heart, studying the mechanisms of blood flow regulation in diabetic animals at rest and under conditions of altered supply and demand would generate more information on the relationship between coronary flow and cardiac function in diabetes. In addition, both primary and secondary complications of diabetes could have a bearing on metabolic control of coronary blood flow, since the release of cardiac metabolites is sensitive to the biochemical processes of substrate utilization, energy production and energy transduction, as well as hemodynamic and neural function.

Currently, the role of adenosine in coronary blood flow regulation is being examined in experimentally diabetic animals. Koltai et al., after observing a decreased coronary hyperemic response in 12 - week diabetic dogs, reported a reduction in dilator response to infused adenosine (1984). Liang and Belloni (1986) infused adenosine into the isolated perfused diabetic rat heart and reported a decreased response to adenosine, but a normal vasodilatory response to nitroprusside, demonstrating that it was the sensitivity to adenosine and not the capacity to vasodilate that was impaired in these rats. Downing infused adenosine into diabetic lamb coronary arteries and also observed a lack of response to the infused adenosine over a range of concentrations (1985). Insulin replacement normalized the dose - response curve to adenosine, raising the possibility that the adenosine receptor responds to adenosine in the presence of insulin. Since insulin alone has been shown to cause

vasodilation in the neonatal lamb coronary vasculature and in the rabbit ear artery (Downing and Lee, 1979; Nelson and Steinsland, 1984), it is possible that insulin enhances the effect of adenosine on vascular smooth muscle by adding to adenosine - mediated vasodilation through its own ability to inhibit calcium channels. Insulin also causes an increase in extracellular hydrogen ion, which is known to increase adenosine receptor sensitivity in both normal and diabetic hearts (Downing et al, 1982; Merrill et al., 1978, Moore, 1983). Thus, there are two possible mechanisms for insulin's effects on adenosine sensitivity. With these observations and the known alterations in autonomic function, there could be changes in the metabolic and neural control of coronary blood flow in diabetes. Furthermore, with the known presence of diabetic neuropathies, there may be neural - metabolic interactions which differ from normal animals.

F. Diabetes and Exercise

Exercise programs are used in conjunction with diet and drug therapy to reduce the hypoglycemia - related symptoms of diabetes. The cardiovascular and metabolic events that occur with both acute bouts of exercise and long - term conditioning programs are currently being evaluated for their benefit to diabetic management.

1. Acute responses to exercise in normal indidivuals

Aerobic exercise is defined as exercise that causes an increase in oxygen consumption (Cooper, 1977). Typical types of aerobic exercise are running, swimming, and bicycling, since when performance is sustained

oxygen consumption increases in individuals performing these exercises. During an acute bout of aerobic exercise there are cardiovascular and metabolic adjustments that allow needed oxygen and nutrients to reach exercising muscle and the brain. The sympathetic nervous system is activated within seconds of exercise initiation, causing an increase in cardiac output through increases in heart rate and stroke volume, an increase in ventilation, and a redistribution of blood flow away from the splanchnic bed. Local metabolic mechanisms cause hyperemia in exercising skeletal muscle and cardiac muscle to match oxygen supply to demand. The intensity of this type of exercise can increase until a maximal level of oxygen consumption is attained. Beyond this level glycolytic energy metabolism exceeds the rate of oxidative metabolism, causing the accumulation of lactic acid and the development of fatigue.

Since ventilation can increase more than enough to allow sufficient alveolar exchange of oxygen and carbon dioxide, the ability to supply the blood with oxygen does not appear to limit aerobic capacity. However, delivery of oxygen to exercising muscle by the cardiovascular system and oxygen utilization by mitochondrial enzymes do reach a maximum and therefore limit maximal oxygen consumption (Lamb, 1984).

Metabolic changes in response to sympathetic stimulation with exercise involve the release of glucose and free fatty acids from hepatic glycogen and adipose triglyceride stores, respectively, due to the release of epinephrine, glucagon, growth hormone, and cortisol. The ratio of glucose/fatty acid utilization in skeletal muscle and myocardial tissue increases with exercise intensity and decreases with exercise duration

(Hagenfeldt, 1979). Glucose transport into skeletal and cardiac muscle is enhanced during and after exercise, even without an increase in insulin release (Ploug et al., 1984). It has been proposed that either insulin binding is facilitated during exercise or there is another mediator of glucose transport released from muscle during contraction. A recent study indicates that insulin binding is probably reduced during exercise, although insulin binding is enhanced after exercise (Koivisto et al., 1979). In addition, the enhanced glucose uptake observed with exercise has been observed in isolated muscle perfused with an insulin - free medium (Richter et al., 1985). Adenosine, which is released from skeletal and cardiac muscle with exercise, has been shown to increase glucose transport in adipose and muscle (Dole, 1962; Londos, 1986). It is possible, therefore, that adenosine may play a role in glucose uptake during exercise.

2. Chronic effects of exercise in normal individuals

After a period of 8 - 12 weeks of regular aerobic exercise, the cardiovascular system and skeletal muscle enzymatic machinery undergo adaptations that allow for an increase in maximal aerobic capacity and therefore maximal level of performance. Fifty percent of the increase in maximal aerobic capacity is due to an increase in cardiac output, and the other fifty percent increase is due to enhanced oxygen extraction across capillaries of exercising muscle (Lamb, 1984).

The cardiovascular changes that occur with training allow cardiac output to increase at a given exercise intensity as compared to the same exercise intensity before training and provide an increase in maximum

cardiac output. Resting heart rate is decreased due to an increase in vagal tone and a decrease in circulating catecholamines, and stroke volume increases due to a combination of a greater ventricular filling time and cardiac hypertrophy, the occurance of which depends on the type of exercise. (Lamb, 1984; Oscai et al., 1971; Stevenson et al., 1969). In rats subjected to chronic exercise cardiac contractile function of isolated hearts has been shown to increase after training but that of in - vivo hearts has not (Schaible and Scheuer, 1979). Since filling pressure and afterload can be controlled in isolated heart preparations, this model may better serve as an indicator of how the heart adapts to stress after conditioning. The observed increased functional capacity of the isolated rat heart with conditioning is associated with enhanced myosin, actomyosin, and myofibril ATPase (Bhan and Scheuer, 1972, Malhotra et al., 1976).

The increase in oxygen extraction in skeletal muscle is attributed to an increase in intracellular oxygen binding and mitochondrial Krebs cycle and electron transport enzymes (Oscai et al., 1971). Skeletal muscle myoglobin, succinate dehydrogenase, the ability to oxidize pyruvate to palmitate and number of mitochondria are elevated in normal animals after exercise conditioning (Lamb, 1984).

Endocrine changes that occur after aerobic training reflect a more efficient use of energy substrates and a reduced hyperglycemic response to the stress of exercise. Insulin secretion during exercise and insulin sensitivity are elevated in trained individuals compared to untrained individuals. Basal circulating catecholamines are reduced and during

exercise the release of epinephrine, norepinephrine, glucagon, growth hormone, and cortisol is reduced as compared to pre - training levels (Brooks and Fahey, 1985). Other metabolic adaptations to exercise training include reduced levels of low density lipoproteins, which are considered deleterious to the coronary vasculature, and increased levels of high density lipoproteins, which are thought to limit the accumulation of cholesterol in coronary arteries (Lamb, 1984).

The effects of exercise training on coronary blood flow regulation have been studied in several animal models. In the isolated perfused rat heart, enhanced coronary dilator capacity was observed, and it was suggested that hearts from conditioned rats would be protected from hypoxia or ischemia (Penpargkul and Scheuer, 1970). However, an in-vivo rat heart preparation studied by Yipintsoi et al (1980) failed to show any advantage in flow maintenance after 15 minutes of hypoxia, although another laboratory using the same model (Carey et al., 1976) reported an increase in flow maintenance of hearts from conditioned rats after 5 minutes of hypoxia. In the conscious dog, coronary blood flow and coronary vascular resistance were found to be unchanged after exercise conditioning (Carey et al., 1983), but Barnard et al. (1980) observed decreases in coronary blood flow at rest and during submaximal exercise after training. The decrease in resting and submaximal coronary blood flow after exercise conditioning is consistant with an enhanced myocardial oxygen extraction. A decrease in myocardial reactive hyperemia was reported by Knight and Stone (1983) after partial conditioning and they suggested that there is enhanced alpha - adrenergic tone after training.

3. Acute responses to exercise in diabetics

As in normal individuals, diabetics respond to an acute bout of aerobic exercise with increases in cardiac output and ventilation. Studies examining the cardiac and skeletal muscle blood flow responses to exercise are lacking. The main concern in the exercising diabetic involves the enhanced glucose transport that occurs during exercise (Brooks and Fahey, 1985). In diabetics receiving insulin, this rise in insulin sensitivity and/or non - insulin mediated glucose transport results in hypoglycemia. This is dangerous because a lack of coordination and judgment can occur with exercise during hypoglycemia, and the patient may not realize that he or she is approaching unconsciousness. Therefore a lower insulin dose or the ingestion of carbohydrates prior to exercise is usually recommended to the diabetic.

4. Chronic effects of exercise in diabetics

The glucose - lowering effect observed in the diabetic with acute exercise has not consistently been associated with better glycemic control in the diabetic after exercise conditioning. Resting blood glucose levels and glycosylated hemoglobin values were reported to be unchanged in Type I diabetic patients subjected to 18 weeks of aerobic exercise (Zinman et al., 1984). In contrast, moderately diabetic streptozotocin - treated rats showed decreased basal glucose levels after a training program with unchanged plasma insulin values (Tancrede et al., 1982). These findings are consistent with the increased insulin sensitivity observed in both Type I and Type II diabetics after exercise conditioning (Richter et al., 1981, 1985).

Several cardiovascular adaptations to chronic exercise documented in normal individuals, such as a reduced resting heart rate and increased maximal oxygen consumption, are observed in diabetic patients as well. Circulating levels of low density lipoproteins, which are elevated in diabetic patients, are reduced in relation to beneficial high density lipoproteins in diabetic subjects as seen in normal subjects after training. Autonomic neuropathy, however, has been shown to be accentuated in some diabetics after exercise conditioning (Bergman and Averhahn, 1985). Thus it is important to evaluate the possible benefits and risks of exercise with regard to the many subtle factors leading to development of long - term diabetic complications.

Experimental animals have been used to evaluate biochemical changes that may occur in muscle with exercise conditioning in diabetics. Cardiac and skeletal muscle enzymes that increase with exercise conditioning in normal animals have been shown to be reduced in sedentary diabetic animals. Exercise training corrects deficiencies in skeletal muscle myoglobin, succinate dehydrogenase and the ability to oxidize pyruvate to palmitate in diabetic rats (Ianuzzo et al., 1974). However, this exercise related improvement in enzyme capacity has not been observed in cardiac muscle. In a study by Belcastro et al. (1985), cardiac myofibril ATPase activity was assessed in normal and diabetic rats after a swimming program. Although normal rats exhibited increased enzyme activities after training, diabetic rats suffered a further decrease in enzyme capacity. Thus exercise training did not seem to improve this index of cardiac function in the severely Type I diabetic model.

Since many of the enzymatic deficiencies observed in experimental diabetes are corrected with insulin replacement, and because insulin sensitivity increases after exercise training, it is possible that the correction of these abnormalities with exercise is due to enhanced insulin sensitivity. The discrepancies between improvement of cardiac vs. skeletal muscle enzyme capacities with exercise could be related to levels of circulating insulin in each experimental model. It has been suggested that a threshold level of insulin must be present in order for enhanced glucose transport to occur after exercise. Thus, in the more severely diabetic animal with a lower circulating insulin level, the benefits of exercise are less pronounced. This theory is in agreement with the observation that better controlled diabetics are benefit more with regard to glycemic control by exercise programs than poorly controlled diabetics (Bergman and Averhahn, 1985).

Although moderate exercise programs under careful monitoring are often prescribed for diabetic cardiac patients, there is still a debate as to whether exercise programs are beneficial or deleterious to diabetic heart conditions, and there is a need for studies on the effects of exercise training on coronary blood flow regulation in diabetes. Thus, in addition to examining how diabetes affects the relationship between adenosine and coronary vascular resistance in the sedentary subject, it is important to determine how the diabetic responds to acute increases in cardiac work and how exercise training may have an effect on the metabolic regulation of coronary blood flow in the diabetic.

II   RATIONALE

The coronary circulation is controlled by the interaction of hemodynamic, neural and metabolic factors. Metabolic mediators predominate in adjusting flow to meet myocardial metabolic needs in the face of changing hemodynamic and neural factors. Adenosine, a breakdown product of adenosine triphosphate, is a potent coronary vasodilator proposed as a major metabolite involved in the regulation of coronary blood flow.

The case for a role of adenosine in the control of coronary vascular resistance has largely been based on the observation that the content of adenosine in cardiac tissue and effluent rises as coronary vascular resistance decreases during conditions where additional oxygen is needed for the maintenance of cardiac contractile function. The inverse correlation between adenosine production and coronary vascular resistance has been observed under conditions of ischemia, hypoxia, exercise, aortic constriction, and sympathetic stimulation.

Diabetes mellitus, an endocrine disorder affecting the metabolism of most body tissues, including vascular, neural and cardiac tissue, is a prominent risk factor in the development of cardiac disease. The well - documented characteristics of diabetic heart disease include macro - and microangiopathies, autonomic neuropathy, and cardiomyopathy. It is also suspected that defects in coronary blood flow regulation could play a role in the pathogenesis of diabetic cardiac disease.

This project was designed to examine the metabolic control of coronary blood flow and to test the hypothesis that the relationship between myocardial adenosine production and coronary vascular resistance is altered in diabetes mellitus. Several questions were addressed in normal and experimentally diabetic animals.

Question 1:

Is the relationship between adenosine production and coronary vascular resistance altered in diabetes mellitus?

The effects of diabetes on myocardial adenosine production and coronary vascular resistance were examined during basal metabolic conditions, during acute increases in cardiac work, and after a chronic exercise program. The question was studied in anesthetized, open chest animals by measuring myocardial adenosine content in streptozotocin - induced diabetic rats and myocardial adenosine release in alloxan - streptozotocin - induced diabetic dogs in relation to hemodynamic parameters including mean arterial blood pressure, heart rate, left ventricular pressure, dP/dt, and coronary blood flow. Basal data were collected in normal and diabetic rats and dogs. Since exercise conditioning is used as a therapeutic method in the treatment of diabetes, an exercise conditioning program was chosen for a portion of the rats to examine what effects an exercise regime may have on the relationship between adenosine and coronary vascular resistance. Basal data were collected in these rats after eight weeks of treadmill running,

which has been shown to be sufficient to cause training effects in both normal and diabetic rats.

In the dogs, after basal measurements were made, ventricular pressure was increased with an intravenous norepinephrine infusion to see if active hyperemia is altered in the diabetic heart. Since adenosine production is normally correlated with metabolic demand through its link with oxygen consumption, myocardial oxygen consumption was also measured in this part of the study to disclose potential effects of diabetes on changes in myocardial oxygen consumption and adenosine production per unit work.

Question 2:

What are the effects of diabetes on the competition between neurally mediated vasoconstriction and metabolically mediation vasodilation during basal metabolic conditions and norepinephrine − stimulated increases in cardiac work?

One of the prime advantages of metabolic regulation of a vascular bed is the ability to vasodilate almost instantaneously in response to increases in oxygen demand, thus balancing the oxygen supply/oxygen demand ratio. If metabolic control is defective other remote mechanisms regulating blood flow would have a more dominant role, and flow may not be matched to metabolism.   In normal animals, an increase in $alpha_1$ − adrenergic vasoconstrictor tone during sympathetic stimulation is associated with an increase in myocardial adenosine

release, which partially buffers the neurally induced vasoconstriction. Conversely, it would be expected that removal of $alpha_1$ - vasoconstriction during sympathetic stimulation should attenuate the increase in adenosine release. In this portion of the study the effects of $alpha_1$ - adrenergic blockade of the coronary vasculature on myocardial adenosine release and coronary vascular resistance was examined in normal and in diabetic dogs, where metabolic regulation may be defective.

Question 3:

In normal and diabetic dogs, what are the effects of acute insulin administration on adenosine production during active and reactive hyperemia and on adenosine function during exogenous adenosine administration?

It has been suggested that insulin may affect adenosine receptor sensitivity. A decrease in adenosine sensitivity might increase basal or stimulated adenosine levels due to a lack of feedback inhibition of adenosine production, that an adequate oxygen supply would provide. On the other hand, an increase in adenosine sensitivity might decrease adenosine production due to an increase in feedback inhibition. In this portion of the study the effect of acute insulin administration on endogenous adenosine release and coronary vascular resistance during basal and norepinephrine - stimulated conditions was examined in normal and diabetic dogs. Hyperemic responses to infused adenosine and a brief coronary occlusion were also compared before and after insulin

administration in normal and diabetic dogs in order to assess the effects of diabetes and insulin on the sensitivity of the coronary circulation to adenosine.

III ANIMAL MODELS

The two animal models used in this project were the
streptozotocin - induced diabetic rat and the streptozotocin/alloxan -
induced diabetic dog. The rat and the dog were chosen for these studies
because they can be made diabetic by chemical means, can easily be
maintained in the diabetic state, and are large enough for the
interventions required to address the hypothesis. The rat served to
answer basic questions concerning the relationship between adenosine and
coronary vascular resistance. The dog, due to its larger heart size and
blood volume, allowed for the collection of additional information on the
effects of diabetes on adenosine release, oxygen consumption, and
adenosine sensitivity.

Chemical induction of diabetes involves pharmacological
destruction of pancreatic beta cells. Therefore, these animals are
representative of juvenile diabetes, and serve as controlled models in
which one defined etiological factor is present and secondary
cardiovascular and neural complications do develop (Mordes and Rossini,
1981). The two drugs most commonly used to induce diabetes in laboratory
animals are streptozotocin and alloxan. The diabetogenic action of
streptozotocin, a N - nitroso derivative of glycosamine, was discovered
by Rakieten, Rakieton and Nadkarni, in 1963. The drug is used at high
doses as a chemotherapeutic agent, but doses between 25 and 100 mg/kg are
specifically toxic to the pancreatic beta cell (Junod et al., 1969).
Serum glucose, urine volume, and glycosurea increase, and serum insulin

and pancreatic insulin decrease in proportion to streptozotocin dosage. The most well accepted mechanism of streptozotocin's action on the pancreatic beta cell involves the depletion of intracellular NAD. In addition, the drug may disrupt the plasma membrane and may act as an oxidant either by increasing the concentration of free radicals or by decreasing beta cell antioxidants (Cooperstein and Watkins, 1981).

Alloxan was shown to produced specific pancreatic beta cell destruction in 1943 by Dunn and McLetchie. At lower diabetogenic doses alloxan is considered to be specific to the beta cell, but kidney and liver damage have been observed with alloxan use in the higher dose range. The primary action of alloxan is on the plasma membrane, and involves the formation of sulfide bonds on cell membranes. Alloxan, like streptozotocin, may also increase intracellular free radicals.

The diabetogenic effects of streptozotocin and alloxan differ among species. For example, the rat is sensitive to both agents, the rabbit is insensitive to steptozotocin, but becomes diabetic with alloxan, and the guinea pig is resistant to alloxan. The differences in specificity may be due to beta cell membrane structure and biochemical differences in the cell. One of the proposed effects of alloxan is chelation of zinc inside the beta cell. Since guinea pig beta cells are zinc deficient, it is possible that their resistance to alloxan is due to the lack of zinc. The dog is sensitive to both drugs, and is prone to developing liver and kidney damage with doses of alloxan that are safe in rodents, so alloxan and streptozotocin are given together in low doses. Multiple low doses of each drug have been attempted, but the

hyperglycemia is only transient and sever hepatotoxicity ensues (Stevenson and Parsons,1981).

Both alloxan and streptozotocin are unstable at physiological conditions. They decompose rapidly at room temperature and neutral pH, with half - lives under 15 minutes (Cooperstein and Watkins, 1981). Lowering the pH to 4.5 and keeping the solutions close to zero degrees centigrade delays chemical decomposition, although the drugs should be administered within 10 minutes after being dissolved. Once injected, there is a triphasic response to both chemicals: 1) hyperglycemia (up to six hours); 2) hypoglycemia (6 to 24 hours) and 3) hyperglycemia, (permanent after 24 hours). The cause of the initial hyperglycemic period is not known, but it is suspected that there may be a decrease in insulin release and a release of catecholamines from the adrenal medulla causing increased glycogenolysis. The hypoglycemic phase is due to a release of intracellular insulin, and animals must be provided with dextrose during this time to prevent death from the hypoglycemia. The final hyperglycemic stage is due to destruction of the pancreatic beta cells, producing hypoinsulinemia.

IV MATERIALS AND METHODS

A. Rat Study

1. Preparation

Sprague – Dawley rats (200g) were divided randomly into normal and diabetic groups. Diabetes mellitus was induced in the diabetic group by streptozotocin injection (65mg/kg, pH 4.5, i.p.) and normal rats were injected with vehicle (citrate buffer, pH 4.5). All rats received glucose in their drinking water (5%) for twenty – four hours post injection to prevent death from hypoglycemia due to the release of pancreatic insulin. Diabetes was confirmed with a positive urine glucose determination two days after the streptozotocin injection. All rats were maintained for eight weeks in an environmentally controlled room with 12 hours of light and 12 hours of darkness, and received a standard rat chow diet ad libitum.

Normal and diabetic rats were further divided into sedentary and exercise conditioned groups. The rats in the exercise groups were put on an eight – week treadmill running program that began with 10 minutes of running on a Quinton rodent treadmill at a speed of 20m/min at a 5% incline, and increased gradually up to 60 minutes of running at a speed of 30m/min at an 8% incline. All exercised rats were run five days per week and the sedentary rats were transported to the treadmill room each day but remained in their cages.

2. Surgery

At the end of eight weeks each rat was fasted overnight and anesthetized with Sodium Pentobarbital (45mg/kg, i.p.). Supplemental anesthesia was administered intravenously in order to maintain a surgical

plane of anesthesia. As shown in Figure 4., a polyethylene catheter was placed in the caudal artery for measurement of arterial blood pressure and withdrawal of blood for plasma glucose, pH, and blood gas (pO_2, pCO_2) concentrations. The rat was placed on a Harvard rodent respirator to maintain a physiological blood gas (pH: 7.4; pO_2: 70 - 100 mmHg; pCO_2: 35 - 45 mmHg) profile and the heart was exposed by sternotomy. A twenty gauge angiocath was inserted into the left atrial appendage for the injection of radiolabelled microspheres, and a twenty gauge needle was inserted into the apex of the left ventricle for the measurement of ventricular pressure and dP/dt. Blood pressures were measured with Statham pressure transducers (model P23D6) placed at the height of the heart, zeroed to atmospheric pressure and recorded on a Hewlett Packard physiograph (model 7758B).

3. Experimental design

After all surgery was completed and blood gasses were normalized, the rat was allowed to stabilize for ten minutes. At the end of this period hemodynamic parameters were recorded (phasic and mean arterial blood pressure, intraventricular pressure, dP/dt) and an arterial blood sample was withdrawn for plasma glucose determination. Coronary blood flow was measured with 15 - micron radioactive microspheres (see Coronary Blood Flow section). The radioactivity (counts per minute) of an aliquot of the microsphere suspension was determined prior to injection on a Searle gamma radiation counter (model 1185). The volume of the microsphere mixture was determined to provide a minimum of 400 microspheres per tissue sample, as recommended by Heymann et al. (1977). The spheres were sonicated for at least 10 minutes and vortexed

Figure 4. Open chest rat model. Hemodynamic parameters (blood pressure, ventricular pressure and dP/dt) were monitored via catheters placed in the caudal artery and left ventricle. Blood gas profile was maintained within a normal range with a ventilator. Coronary blood flow is determined with a left atrial injection of radioactive microspheres. Heart was excised *in - situ* with nitrogen frozen tongs for adenosine determination.

MICROSPHERES

Left ventricular pressure
dP/dt

arterial blood pressure
blood withdrawal
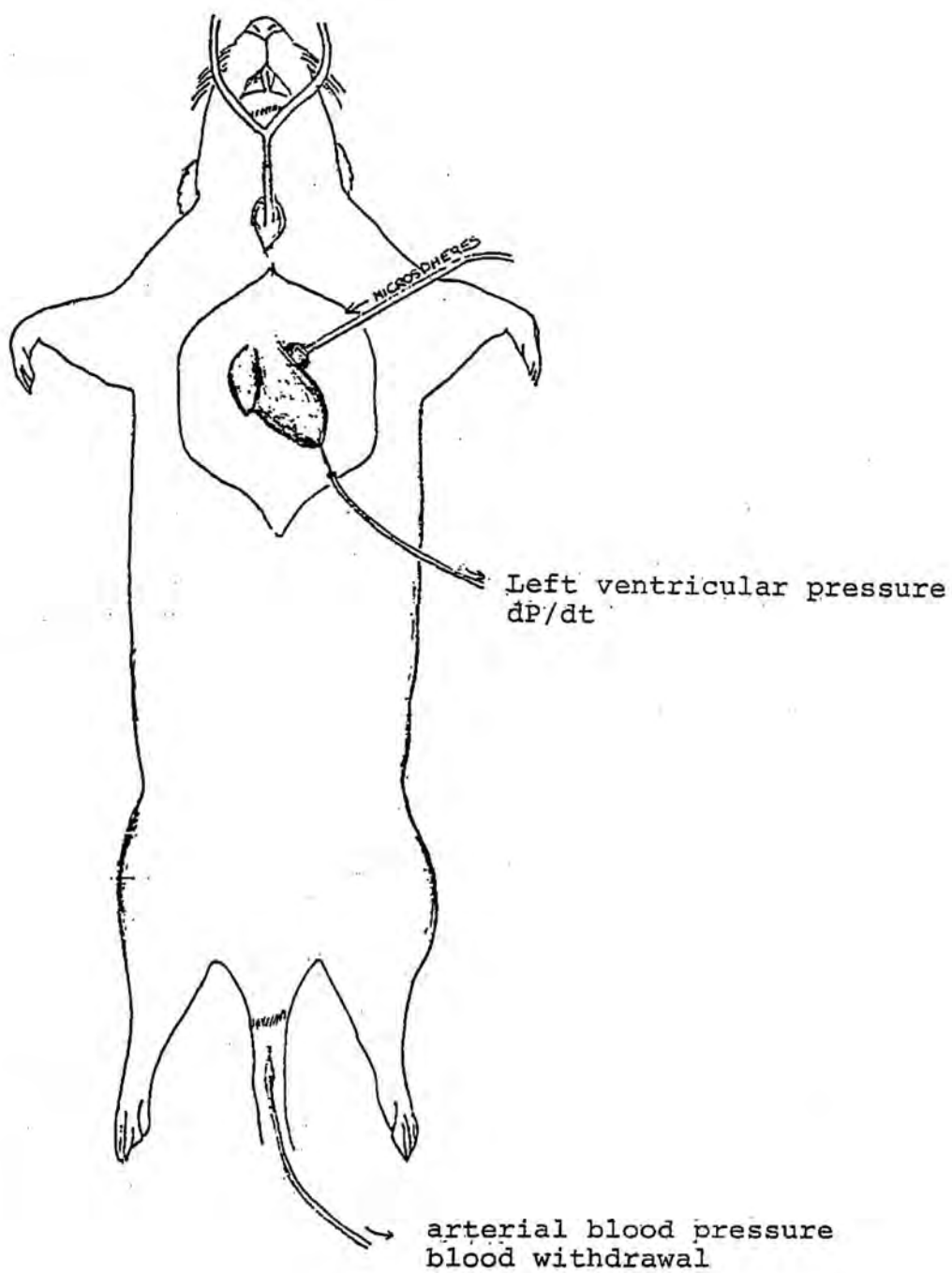
vigorously just prior to the left atrial injection. Care was taken to inject the microspheres slowly in order to prevent any changes in ventricular pressure. An arterial reference sample was taken at a rate of 1 ml per minute during the microsphere injection until one minute past the end of the injection. The microsphere injection syringe was flushed with 1 ml of rat blood from a donor of the same group in order to maintain blood pressure during the blood withdrawal. After the microsphere administration the heart was rapidly clamped *in - situ* with Wollenburger tongs precooled to the temperature of liquid nitrogen and quickly plunged into a container of liquid nitrogen. On the same day the heart was prepared for adenosine and lactate determination by acid extraction as described in the Chemical Analysis section. Only hearts clamped in less than three seconds, and which were flattened into a wafer less than or equal to 2mm thick were used in the study in order to prevent metabolic changes that could occur with a delay in cooling time. After chemical preparation, the precipitate of the tissue homogenate was analyzed for radioactivity and blood flow calculation. Both kidneys were removed and counted to confirm adequate mixing of microspheres and minimal microsphere streaming.

B. Dog studies

1. Preparation

Young mongrel dogs (1 - 5 years of age, weighing 15 - 22 kg) of either sex were divided into normal (n =10) and diabetic (n = 6) groups. The dogs were vaccinated for rabies and distemper and were free of heartworms and other parasites. Induction of diabetes was according to the method of Stevenson and Parsons (1981) as follows: After a sixteen -

hour fast, each dog received an intravenous injection of 20% mannitol (0.5 g/kg) to induce diuresis. Twenty minutes later, a diabetogenic injection of streptozotocin (30 mg/kg) and alloxan (50 mg/kg) in saline (pH 4.5) was administered intravenously. Since pancreatic beta – cell lysis occurs approximately eight hours post injection, 500 ml of 10% dextrose was given subcutaneously to prevent death from hypoglycemia. Diabetes was confirmed by a non – fasting plasma glucose concentration of at least 200 mg/100 ml after four days. The dogs were maintained for ten weeks on a standard dog diet and canned food as needed to preserve body weight. Insulin was supplemented as needed to regulate plasma glucose levels between 200 and 400 mg/100ml plasma and to prevent ketoacidosis. No insulin was given within one week of the experimental procedure.

2. Surgery

On the day of the experiment a fasted dog was anesthetized with Sodium Pentobarbital (30 mg/kg, i.v.). Supplemental doses were given to maintain a surgical plane of anesthesia. Figure 5. illustrates the surgical preparation described below. Under fluoroscopy, a Sones coronary sinus catheter was introduced into the coronary sinus from the left jugular vein for the withdrawal of coronary sinus blood, and a Millar double pressure – tip transducer was placed in the left ventricle via the left femoral artery in order to record left ventricular and arterial blood pressures and cardiac dP/dt. Polyethylene catheters were placed in the left femoral vein and right brachial artery for drug administration and blood collection, respectively. Electrocardiography leads were placed subcutaneously in order to monitor cardiac electrical activity. A bilateral vagotomy was performed to prevent reflex

bradycardia during norepinephrine stimulation of the heart. The dog was ventilated with a Harvard canine respirator to maintain a physiological blood gas (pH: 7.4; pO_2: 70 - 100 mmHg; pCO_2: 35 - 45 mmHg) profile. A positive end expiratory pressure of 3 - 8 cm H_2O was used to prevent atelectasis.

The heart was exposed by left lateral thoracotomy, the cranial lobe of the left lung was wrapped in moist gauze and secured outside the thorax. The heart was suspended in a pericardial cradle. A 20 gauge angiocath was inserted into the left atrial appendage for the delivery of 15 - micron radiolabelled microspheres (see Coronary Blood Flow section). The left anterior descending coronary artery (LAD) and left main coronary artery were isolated for the placement of an electromagnetic flow probe and for injection of drugs, respectively. In the "Insulin Replacement Study" only, a Herd - Barger catheter was inserted into the LAD just proximal to the electromagnetic flow probe in order to selectively infuse drugs into the LAD.

Periodic samples of anaerobically collected arterial blood were taken in order to monitor and correct pH, pO2, and pCO2. Intravenous saline was administered to maintain hydration if necessary. When all surgery was completed and arterial blood gases were normal (ph 7.4, PO2 100, PCO2 40), a 15 - minute stabilization period was allowed prior to the experimental procedure and data collection.

3. Experimental design

Both normal and diabetic dogs were studied under three treatment conditions: a. Control; b. Alpha - adrenergic Blockade; c. Acute Insulin Administration. In each treatment the following procedure was

Figure 5. Canine heart preparation. Catheters were placed in the coronary sinus, left atrium, aorta and left ventricle. In the prazosin study, a needle was placed in the left main coronary artery for injection of drugs and an electromagnetic flow probe was placed on the LAD. In the insulin administration study, a Herd - Barger catheter was secured in the LAD proximal to an electromagnetic flow probe.
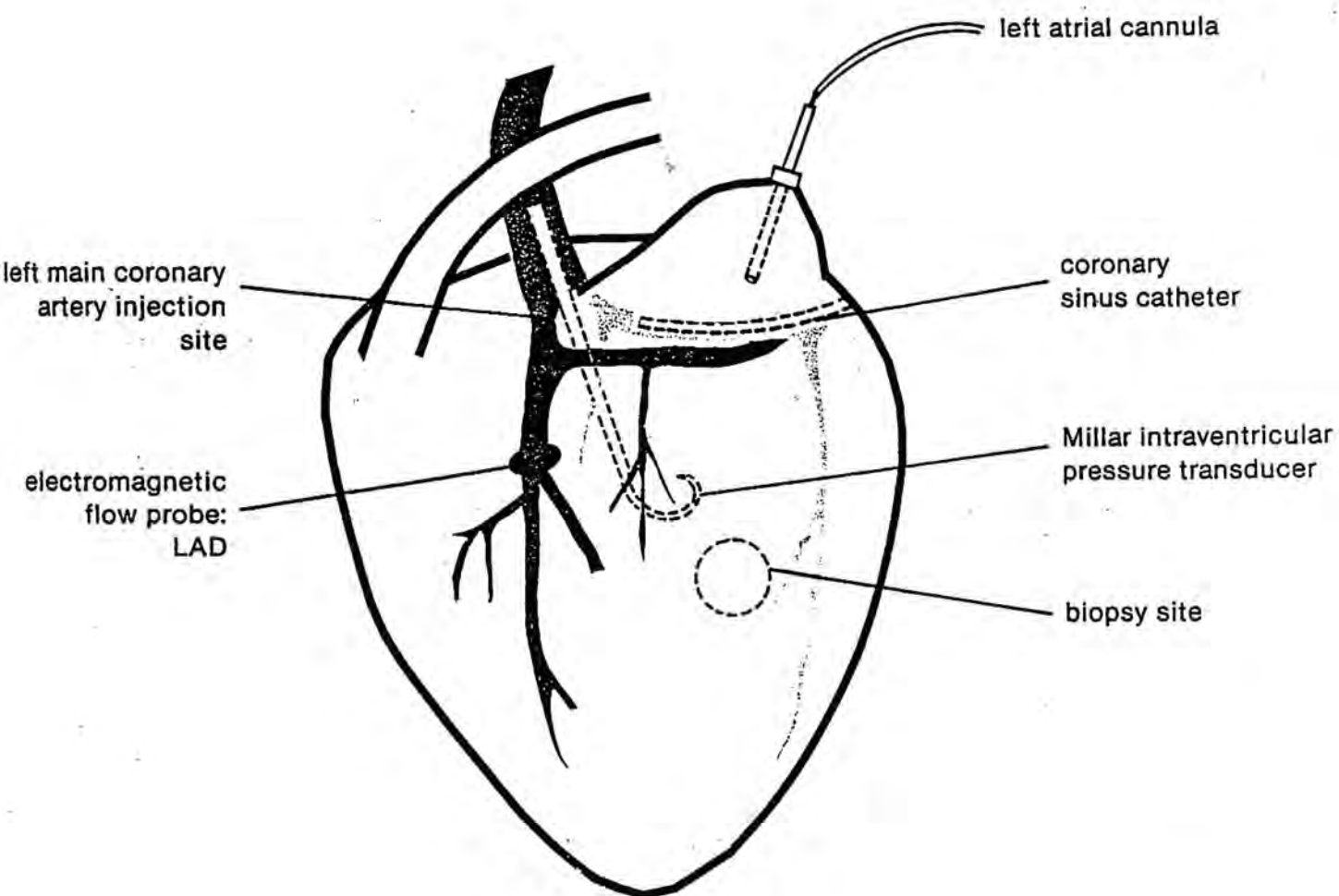
Open-chested dog heart model

followed: Phasic and mean arterial blood pressure, intraventricular pressure, dP/dt, phasic and mean coronary blood flow and an electrocardiogram were monitored on a Gould biological recorder. Arterial and coronary sinus blood were withdrawn simultaneously for the measurement of pH, PO2, PCO2, oxygen, glucose, lactate and pyruvate. Regional coronary blood flow was measured with 15 - micron radiolabelled microspheres. As described in the Coronary Blood Flow Determination section, a precalculated aliquot of the microsphere suspension allowing a minimum of 400 counts per minute of radioactivity per sample was sonicated for 40 minutes, vortexed thoroughly, and injected into the left atrial catheter. An arterial reference sample was withdrawn from the start of the microsphere injection to one minute past the end of the injection at a rate of 2 ml per minute. Two mls of saline were used to flush the microsphere injection syringe during the arterial withdrawal. A different radionuclide was chosen at random for each treatment. Following the final microsphere injection, the dog was terminated with an overdose of Sodium Pentobarbital. The heart and kidneys were removed and placed in formaldehyde in preparation for blood flow determination and verification of microsphere distribution, respectively. After two days, the organs were sectioned, weighed and counted for radioactivity.

a. Control Study

1. Basal state: Myocardial adenosine production, myocardial oxygen consumption, coronary blood flow, coronary vascular resistance, and ventricular performance were measured in both normal and diabetic dogs in the basal state.

2. Sympathetic stimulation: Myocardial adenosine production, myocardial oxygen consumption, coronary blood flow, coronary vascular resistance, and ventricular performance were evaluated with sympathetically mediated increased metabolic demand. Ventricular work was increased by an intravenous infusion of norepinephrine (0.1 mg/ml). The infusion rate was gradually raised until ventricular pressure increased by approximately 70%. A steady - state condition was maintained for ten minutes before data collection.

b. Alpha$_1$ - adrenergic Blockade Study

1. Basal state: Alpha$_1$ - adrenergic coronary tone was removed in order to assess its effects on adenosine release and coronary vascular resistance, and to compare the degree of alpha$_1$ - adrenergic tone between normal and diabetic dogs. Prazosin (0.5 mg), an alpha$_1$ - adrenergic blocking agent, was injected directly into the left main coronary artery with a 27 gauge needle. The blockade was verified by measuring coronary blood flow responses to an intracoronary injection of phenylephrine, an alpha$_1$ - receptor agonist. Data was collected within twenty minutes of the prazosin injection.

2. Sympathetic stimulation: The influence of alpha$_1$ - adrenergic activity on myocardial adenosine release and coronary vascular resistance during a sympathetically - mediated increase in cardiac work was evaluated in normal and diabetic dogs. Alpha$_1$ - adrenergic coronary tone was removed with an intracoronary injection of prazosin (0.5 mg). Within 20 minutes, ventricular pressure was increased by 70% with an intravenous infusion of norepinephrine as described above.

c. <u>Acute Insulin Administration Study</u>

1. Basal state: Myocardial adenosine production, myocardial oxygen consumption, coronary blood flow, coronary vascular resistance, ventricular performance, coronary reactivity to adenosine and reactive hyperemia were studied before and after an acute dose of insulin. Regular porcine insulin (E. Lilly) was administered intravenously at a dose (1.5 units/kg) commonly used to lower blood glucose to normal levels in chemically diabetic dogs. In order to assess coronary reactivity, adenosine was infused into the LAD at doses of 18, 37, 74, and 111 nmoles, and peak coronary flow was measured with an electromagnetic flow probe on the LAD. Reactive hyperemia was assessed after a 5 - second occlusion of the LAD.

2. Sympathetic stimulation: Myocardial adenosine release, myocardial oxygen consumption, coronary blood flow, coronary vascular resistance, and ventricular performance were evaluated with sympathetically - mediated increased metabolic demand. After the acute insulin administration (Section 3.A.) ventricular pressure was increased by 70% with an intravenous infusion of norepinephrine as described above. A steady - state condition was maintained for ten minutes before data collection.

C. <u>Coronary Blood Flow Determination</u>

Radioactive microspheres, carbonized beads uniformly labelled with radioactive isotopes and coated with a polymeric resin, used in this

study were 15 ± 3 microns in diameter and were labelled with Cerium [141], Strontium [85], Scandium [46] (3M Company, St. Paul, Mn.), Tin [113], and Niobium [95] (New England Nuclear, Boston, Mass.). Microspheres were stored suspended in sterile 10 % dextran with an initial activity of 1 mCi per 10 ml suspension.

The theory of microsphere use is based on their rheological similarities to red blood cells. Once injected into the heart where they are mixed, microspheres are evenly distributed into the bloodstream and become trapped in the microcirculation on the first pass through the body. The addition of 0.001% detergent Tween 80 into the dextran vehicle prevents microsphere clumping during storage, and the suspension is also sonicated and vortexed prior to the injection to allow even dispersal. Dispersement is checked by microscopic examination after sonication, and uniform mixing in blood is evaluated by comparing blood flows in paired organs, such as the kidneys. The amount of microsphere suspension injected is determined by the radioactivity of the isotopes and the size of the sample to be counted. Error is minimized when at least 400 microspheres are trapped in each sample, and there are at least 4000 counts per minute per sample (Heymann et al., 1977).

In order to calculate organ blood flow using microspheres an arterial reference sample must be established. During the microsphere injection, blood is withdrawn at a known rate. The withdrawal is continued for one minute, until all microspheres are cleared from the arterial circulation and embedded in capillaries. The reference sample counts, withdrawal rate, sample counts and sample weight are used as follows to determine tissue blood flow:

$$\text{Tissue flow} = \frac{\text{Tissue counts per minute X Withdrawal rate}}{\text{Withdrawal counts per minute}}$$

Tissue vascular resistances are derived by dividing the mean arterial pressure at the time of injection by tissue flow. Cardiac output was calculated by the following formula:

$$\text{Cardiac output} = \frac{\text{Total counts per minute injected x Withdrawal rate}}{\text{Withdrawal counts per minute}}$$

Since several isotopes are often used in one tissue sample, pure samples of each isotope are counted to determine backscatter. The backscatter is then subtracted from each mixed tissue sample.

D. Chemical Analysis

    1. Rat Study

        a. Blood Analysis

            1. Blood gasses

Arterial blood (0.1 ml) was collected anaerobically in heparinized glass hematocrit tubes. PO_2, PCO_2, and pH were measured on a Radiometer BMS3MKA blood gas analyzer (Radiometer/Copenhagen, Cleveland, Ohio).

            2. Plasma glucose

Arterial blood (0.1ml) was collected in heparinized glass

hematocrit tubes. After centrifugation the plasma (0.01 ml) was analyzed for glucose concentration on a Beckman Glucose Analyzer (Beckman Instruments, Puerto Rico) using the glucose oxidase method. An oxygen - sensitive electrode measured oxygen consumption as the following reaction proceeded:

$$\text{Glucose + Oxygen} \xrightarrow[\text{water}]{\text{glucose oxidase}} \text{Gluconic acid + Hydrogen peroxide}$$
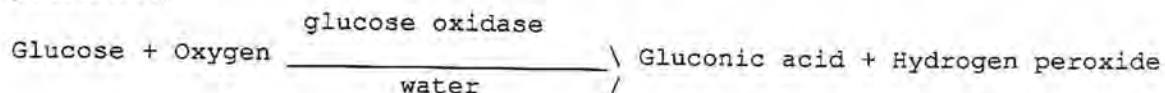
b. Tissue Analysis

The frozen clamped heart was removed from the liquid nitrogen and placed into a steel cylinder precooled to the temperature of liquid nitrogen. The sample was pulverized with a steel rod into a fine powder. The powder was placed in a nitrogen - frozen glass tube, weighed, and poured into a plastic tube cooled in dry ice. Perchloric acid (0.5 N, 5 ml/g tissue) was added to the sample in order to denature proteins. The mixture was homogenized in a Polytron homogenizer for 10 seconds and centrifuged at 10,000 g for 20 minutes. The supernatant was neutralized with potassium hydroxide and the tissue precipitate was transferred to a counting tube and analyzed for radioactivity for blood flow determination.

1. Adenosine

The neutralized tissue extract was centrifuged at 10,000 g for 10 minutes. The volume of the supernatant was determined for quantitation of adenosine. A 0.4 ml aliquot was added to 0.8 ml of a 50 mM phosphate buffer containing EDTA (pH 7.6). The solution was filtered through a Millipore filter (0.2 micrometer pores) and poured into glass microcuvettes.

Adenosine was measured in a Perkin - Elmer dual - beam, dual - wavelength spectrophotometer by conversion to uric acid (Olsson, 1970). Since the extract contained hypoxanthine and inosine, as well as adenosine, a stepwise conversion of the three metabolites was performed. In the first reaction, hypoxanthine was converted to uric acid with the enzyme, xanthine oxidase. After the reaction ran to completion, a second enzyme, nucleoside phosphorylase, was added to the cuvette to convert inosine to hypoxanthine and then to uric acid by the xanthine oxidase already present. In the third reaction, adenosine deaminase was added to the cuvette to convert adenosine to inosine, followed by conversion to hypoxanthine and to uric acid (see Figure 3). Absorbance changes signifying conversion to uric acid were determined at a wavelength of 292 nm. After a stable baseline was established signifying the end of the reaction, adenosine content was calculated as follows:

$$\text{Adenosine (nmoles/g)} = \frac{\text{Change in absorbance}}{\text{Extinction coefficient (11.6)}} \times \frac{1.2 \text{ ml}}{0.6 \text{ ml sample}} \times \frac{\text{Sample volume}}{\text{Sample weight}}$$

2. Dog Study

   a. Blood Analysis

      1. Blood gases

Arterial and coronary venous blood (2ml) were collected anaerobically in cold, heparinized syringes. PO_2, PCO_2, pH and oxygen content were measured on a Radiometer ABL - 3 blood gas analyzer

(Radiometer/Copenhagen, Cleveland, Ohio). Oxygen consumption (ml O_2 · min^{-1} · $100g^{-1}$) was expressed as the product of coronary blood flow and the difference between arterial and venous oxygen concentration.

2. Plasma glucose

Venous blood (1 ml) was collected in heparinized tubes and centrifuged for 10 minutes at 10,000 g. Plasma (0.01 ml) was analyzed as described previously for glucose concentration.

3. Glycosylated hemoglobin

An aliquot of venous blood (0.05 ml) was hemolyzed and fast (glycosylated) hemoglobin was separated from slow (unglycosylated) hemoglobin on an Isolab Code QS9100 hemoglobin column. The fast hemoglobin and total hemoglobin concentrations were measured spectrophotometrically at a wavelength of 415 nm. Hemoglobin glycosylation is expressed as a fraction of total hemoglobin.

4. Adenosine

Plasma adenosine concentration was determined from 1.5 ml of blood collected in ice cold syringes filled with 3 ml dipyridamole (40mM, pH 7.4). The blood and dipyridamole were mixed thoroughly, put into cold tubes and centrifuged at 10,000 g for 20 minutes. The supernatant was added to 3 ml cold perchloric acid (0.5 N) for protein denaturation and adenosine extraction, and the mixture was centrifuged at 10,000 g for 20 minutes. The supernatant was removed and the precipitate was washed with 0.5 N perchloric acid and recentrifuged. The combined supernatants were neutralized to pH 8.0 with potassium hydroxide and recentrifuged.

Ten mg of Dowex AG 1 - 8X (Bio - Rad Laboratories, Richmond, Cal.), an anion exchange resin, were added to each supernatant in order

to purify the sample for high pressure liquid chromatography. The dowexed sample was vortexed vigorously for two minutes and centrifuged to remove the dowex particles containing impurities. The volume of the supernatant was measured in order to determine the dilution of the original sample. One ml of the supernatant was evaporated to dryness and resuspended in 0.2 ml distilled water. The concentrated sample was centrifuged through a Millipore filter (0.2 mm pores) into a collection tube and pipetted into a chromatography vial. By a modification of the method of Klabunde et al.(1979), adenosine concentration was determined by high pressure liquid chromatography. An aliquot (0.04ml) of the concentrated plasma sample was injected onto a reverse phase C_{18} analytical column (microsorb, Rainin Instrument Co.) using a Waters Wisp 710B injector. The column length and internal diameters were 25 cm and 4.6 cm, respectively. The sample was eluded over a 40 - minute period using a nonlinear gradient, beginning with a 90% solution of 20mM KH_2PO_4 and a 10% solution of 25% aqueous methanol, and ending with a 100% solution of 25% methanol. The solvents were pumped through the column with Waters Models 6000A and M-45 chromatography pumps at a constant flow rate of 0.8 ml/min. Adenosine in the eluate was detected by a flow - through cell on a Waters Lambda-Max Model 480 LC Spectrophotometer, and the ultraviolet absorbance at 260 nm was recorded on a Perkin-Elmer Model 023 recorder. Peak area was calculated from the spectrophotometer using a Hewlett Packard Model 3390A integrator. Adenosine concentration was derived by comparing retention time, peak height and area with those of

adenosine standards. The sensitivity of this method is 10 picomoles of

adenosine.

5. Lactate and Pyruvate

Plasma lactate and pyruvate were measured according to the Sigma

method (Sigma Chem. Co., St. Louis, Mo.). This method is based on the

reversible reaction between lactate and pyruvate, catalyzed by the

enzyme, lactate dehydrogenase (LDH), as shown in the reaction below:

```
                         LDH
    Lactic Acid /·_____\ Pyruvic Acid
                \                 /
                  NAD  /___\ NADH
                       \   /
```
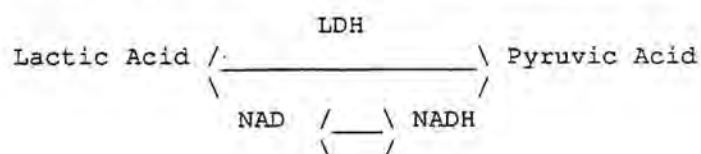
One ml of blood was added directly into 2 ml of cold 8% perchloric acid.

The mixture was vortexed immediately and centrifuged for 10 minutes at

10,000 g.

For lactate determination a 0.05 ml aliquot of the acid extract

was added to a mixture of NAD and LDH in glycine buffer. The sample was

incubated for 30 minutes until the reaction was completed. The formation

of NADH from NAD was detected spectrophotometrically as an increase in

absorbance at 340 nm. The following formula was used to calculate blood

lactate concentration:

$$\text{Blood lactate (micromoles/l)} = \frac{A\ (340)\ X\ 3}{6.22\ X\ 0.0667} = A(340)\ X\ 7.13,$$

where A (340) = Absorbance at 340 nm

      6.22 = Molar extinction coefficient of NAD

         3 = Reaction volume

   0.0667 = Volume of blood

For pyruvate determination, 0.4 ml of the acid extract was addded

to 0.2 ml of a solution containing trizma base and NADH. The absorbance
at 340 nm was measured, and then 0.015 ml of LDH was added to begin the
reaction of pyruvate to lactate. The samples were read on the
spectrophotometer at 2 and five minutes after the enzyme was added to
assure reaction completion. The following formula was used to calculate
blood pyruvate concentration:

$$\text{Blood pyruvate (micromoles/l)} = \frac{\Delta \text{ A (340) X 3}}{6.22 \text{ X } 0.667} = \Delta \text{ A (340) X 7.23,}$$

where Δ A (340) = Initial - final absorbance
            3 = Reaction volume
        0.667 = Vol. blood in sample.

E. <u>Data Analysis</u>

    In the rat study differences within groups were analyzed by
analysis of variance and Duncan's multiple range test. In the dog study,
differences within groups were analyzed by paired T - tests and
differences between groups were analyzed by unpaired T - tests. Linear
regression was used to analyze linear relationships while parabolic
regression was used to analyze curvilinear relationships. Analysis of
Covariance was used to determine differences between two linear
relationships. Differences were considered to be statistically
significant when $p < 0.05$.

V RESULTS

A. Rat Study

1. Hemodynamic data

Table 1 summarizes the effects of diabetes and a running program on hemodynamic parameters. There were no significant differences ($p < 0.05$) in mean arterial blood pressure, heart rate, or cardiac output between sedentary control (SC, $n = 14$), sedentary diabetic (SD, $n = 12$), running control (RC, $n = 12$), and running diabetic (RD, $n = 12$) groups. Left ventricular pressure (LVP), dP/dt (Figure 6), and left ventricular pressure - rate - product (PRP) were significantly reduced in both diabetic groups as compared to control groups. Coronary blood flow (CBF) was reduced in the SD as compared to the SC groups, and CBF decreased in both RC and RD animals. There were no differences in coronary vascular resistance (CVR) between SC and SD groups, and CVR was elevated in RC and RD groups (Figure 7a).

2. Blood data

Plasma glucose concentrations (mg/100 ml plasma) in control and diabetic animals were 94 ± 9.6 and 336 ± 18.5, respectively. There were no differences in blood pH between control (7.47 ± 0.02) and diabetic (7.47 ± 0.02) groups. Because there were no differences in blood data between sedentary and exercise groups in control and diabetic animals, sedentary and exercise blood data were pooled.

3. Adenosine data

As shown in Figure 7b, myocardial adenosine content (ADOC, nmoles/g) was significantly increased in the SD group (8.8 ± 0.68), RD group (9.46 ± 2.4), and RC group (11.48 ± 2.16) as compared to the SC

TABLE 1

HEMODYNAMIC DATA

RAT STUDY

	SC (n = 14)	SD (n = 12)	RC (n = 12)	RD (n = 12)
MABP (mmHg)	107.1 ± 2.96	98.75 ± 4.93	104.0 ± 4.06	96.7 ± 4.86
HR (bpm)	412.5 ± 16.9	372.7 ± 16.4	376.1 ± 11.0	328.1 ± 10.7
LVP (mmHg)	155.7 ± 5.9	123.0 ± 7.6*	146.1 ± 6.9	120.4 ± 5.5*
dPdt $(mmHg/sec^2)$	5557 ± 556	4583 ± 294*	5982 ± 268	4432 ± 345*
PRP (mmHg·bpm)	64712 ± 5470	45341 ± 3907*	54967 ± 3139	39782 ± 2995*
CO (ml/min)	110 ± 5	135 ± 33	110 ± 13	103 ± 25
CBF $(ml·min·100g^{-1})$	514.8 ± 53.5	416 ± 54.7*	332 ± 42.2†	232.1 ± 32.9*†
CVR $(mmHg·min·ml^{-1}·100g^{-1})$	0.263 ± 0.035	0.252 ± 0.02	0.409 ± 0.07†	0.575 ± 0.132*†

values expressed as mean ± SEM
* $p < 0.05$ (compared to same treatment in normal rats)
† $p < 0.05$ (compared to sedentary rats of same group)
SC = Sedentary Control   SD = Sedentary Diabetic
RC = Running Control   RD = Running Diabetic

Figure 6. Effects of diabetes and exercise conditioning on left ventricular pressure (a) and dP/dt (b). Values are expressed as mean ± SEM for sedentary control (SC), sedentary diabetic (SD), running control (RC) and running diabetic (RD) rats. *Denotes significant difference (p < 0.05) from same treatment in control (normal) rats.

*p < .05
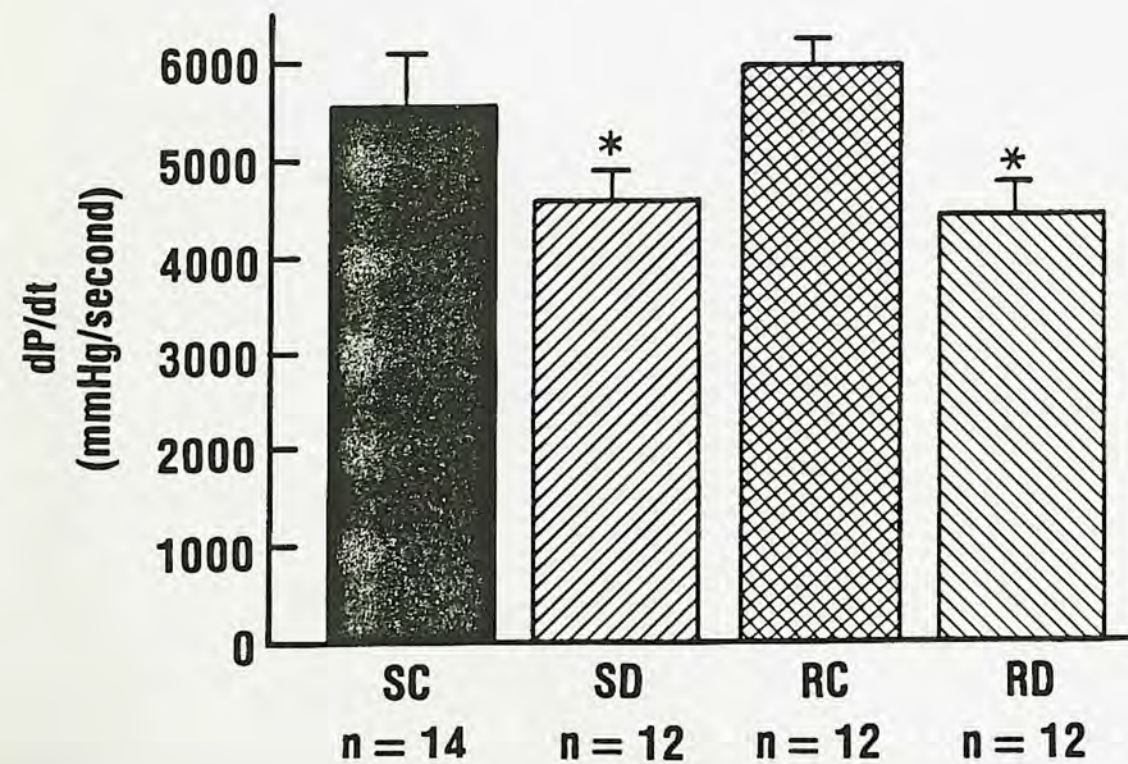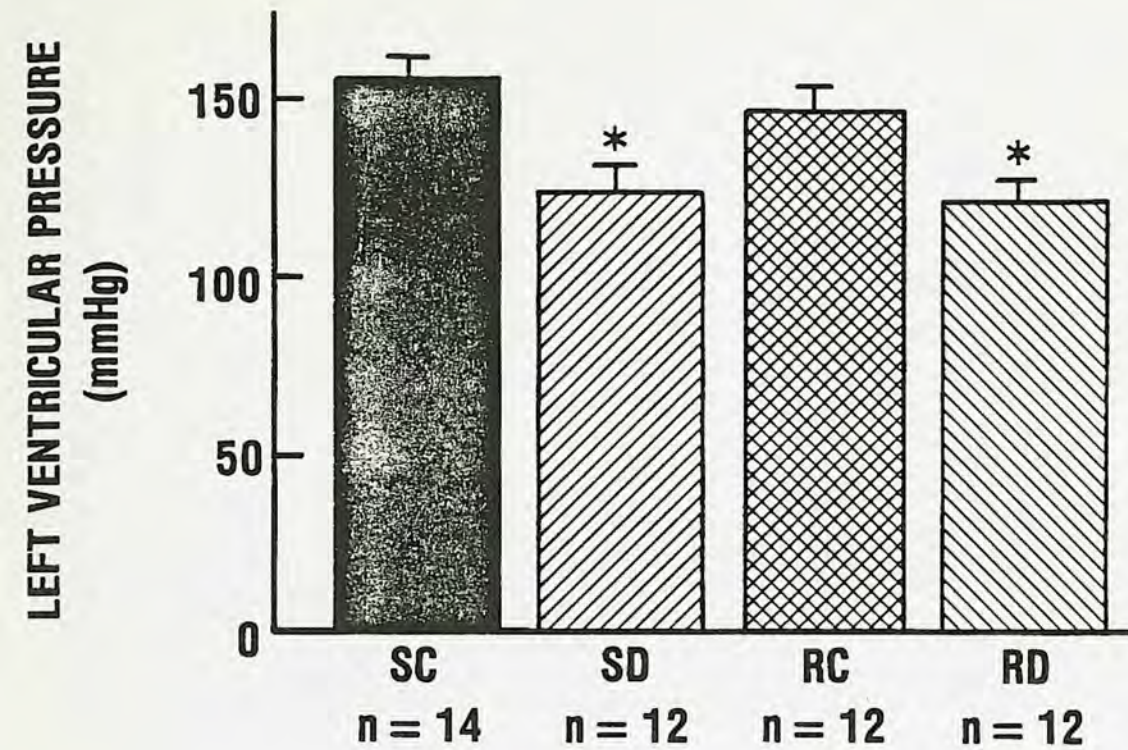
Figure 7. Effects of diabetes and exercise conditioning on coronary vascular resistance (a) and myocardial adenosine content (b). Values are expressed as mean ± SEM for sedentary control (SC), sedentary diabetic (SD), running control (RC), and running diabetic (RD). *Denotes significant difference ($p < 0.05$) compared to same treatment in control (normal) rats. †Denotes significant difference ($p < 0.05$) compared to sedentary rats of same group.
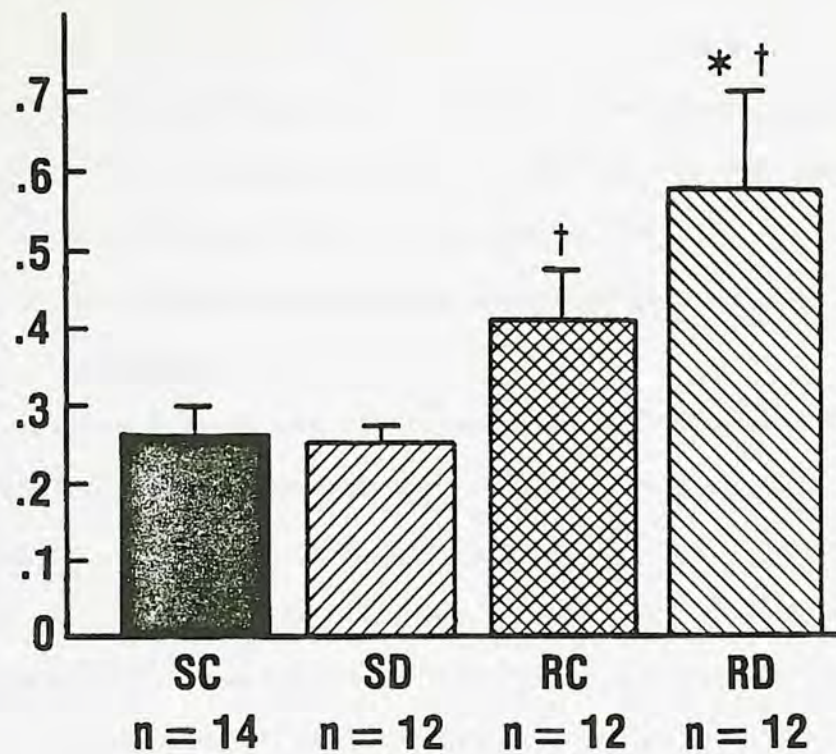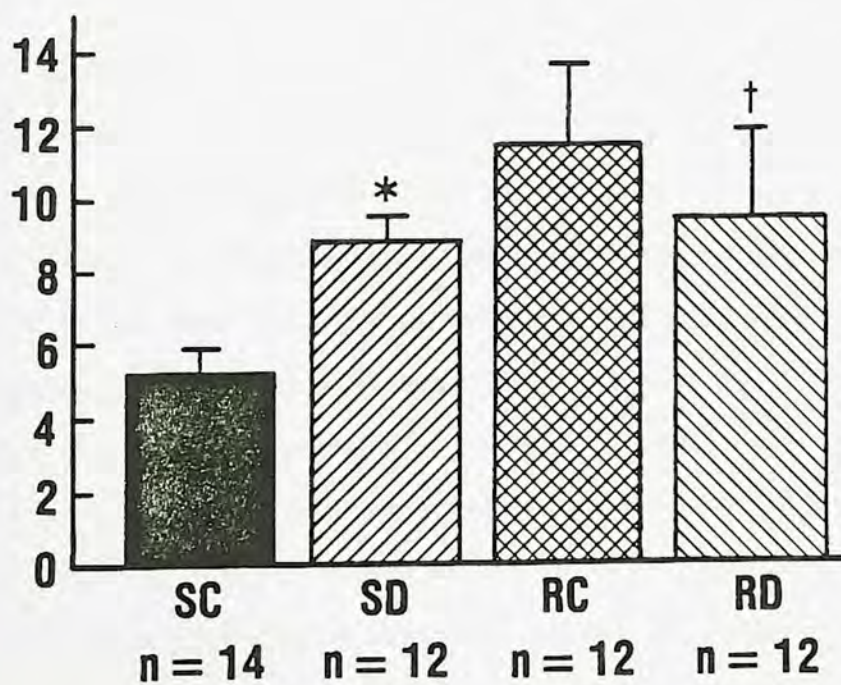
* † p < .05

group (5.23 ± 0.68). Exercise conditioning caused a significant increase in ADOC only in control rats (11.48 ± 2.16). There was no difference in ADOC between SD and RD groups. Therefore, adenosine content was increased in the SD groups without a difference in CVR, and was elevated in the RC and RD groups with an increase in CVR.

4. The relationship between adenosine and coronary vascular resistance

Figure 8 shows the relationship between ADOC and CVR for each rat in SC and SD groups, respectively. There was a significant negative correlation ($r = -0.90$, $p < 0.05$)) between ADOC and CVR in the SC rats, but not in the SD rats ($r = 0.37$, $p > 0.05$). As shown in Figure 9, exercise conditioning caused an increase in adenosine with respect to CVR in the control animals, shifting the curve upwards. After the running program there was a significant negative correlation ($r = -0.81$) between ADOC and CVR in the diabetic rats, as shown in Figure 10.

B. Dog Studies

1. Control Study

a. Hemodynamic data

Table 2 shows hemodynamic parameters for normal dogs at rest (NC, $n = 10$), normal dogs with norepinephrine - mediated sympathetic stimulation (NNE, $n = 10$), diabetic dogs at rest (DC, $n = 6$), and diabetic dogs with norepinephrine - mediated sympathetic stimulation (DNE, $n = 6$). Resting mean arterial blood pressure (MABP) was significantly ($p < 0.05$) higher in the diabetic dogs than normal dogs, and sympathetic stimulation caused an increaase in MABP in both normal and diabetic animals. Heart rate was elevated in diabetic dogs as

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Figure 8. Relationship between myocardial adenosine content and coronary vascular resistance in sedentary control (a, n = 14) and sedentary diabetic (b, n = 9) rats. Values are plotted for each rat. There is a significant correlation (r = 0.90, p < 0.05) between myocardial adenosine content and coronary vascular resistance in the normal rats, but not in the diabetic rats (r = 0.37, p > 0.05).
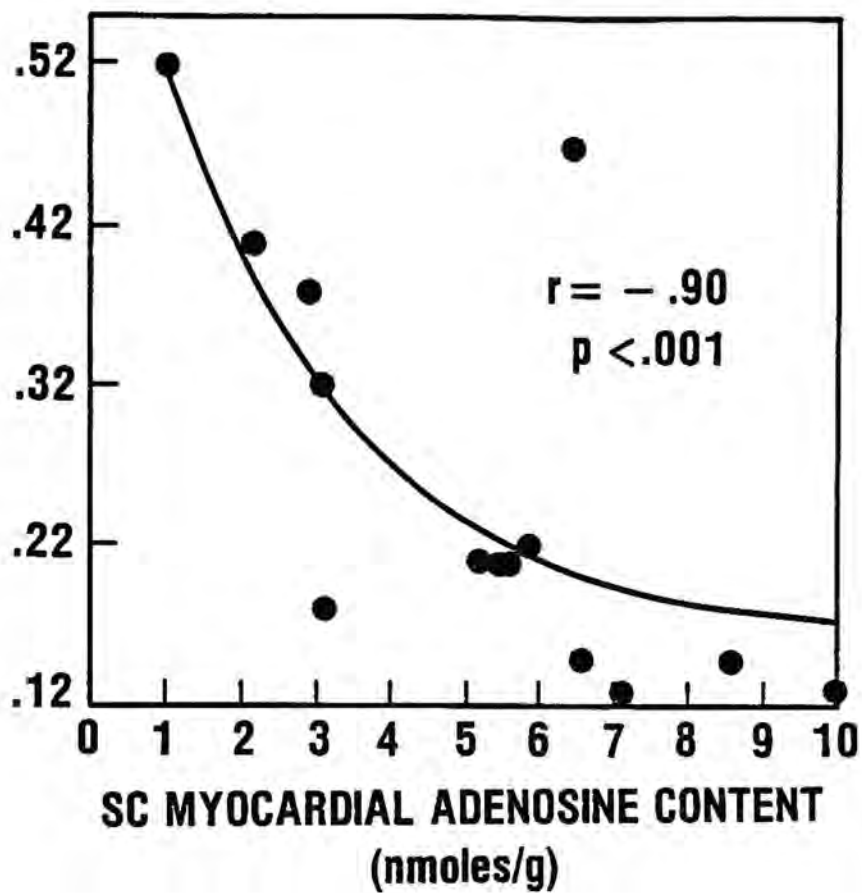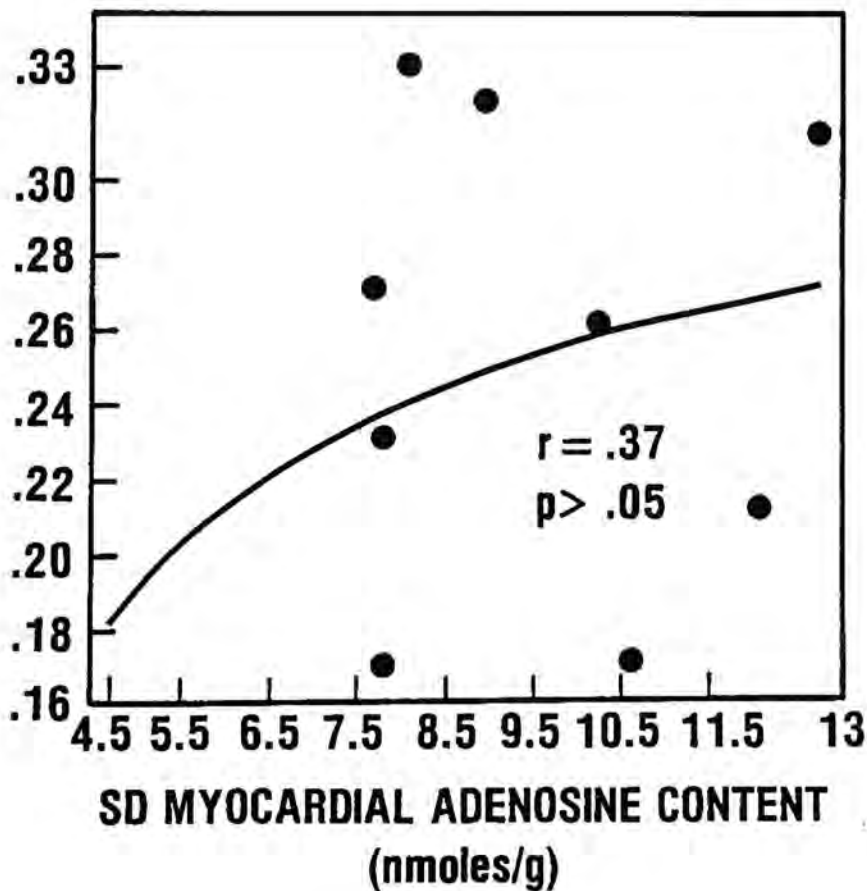
Figure 9. Effects of exercise training on the relationship between myocardial adenosine content and coronary vascular resistance in normal rats. Values are plotted for each rat. Circles denote sedentary control rats and squares denote running control rats. There are significant correlations between myocardial adenosine content and coronary vascular resistance in both sedentary control ($r = 0.90$, $p < 0.05$, $n = 14$) and running control ($r = 0.81$, $p < 0.05$, $n = 12$) groups. Exercise training caused a significant ($p < 0.05$) increase in resistance with respect to adenosine content.
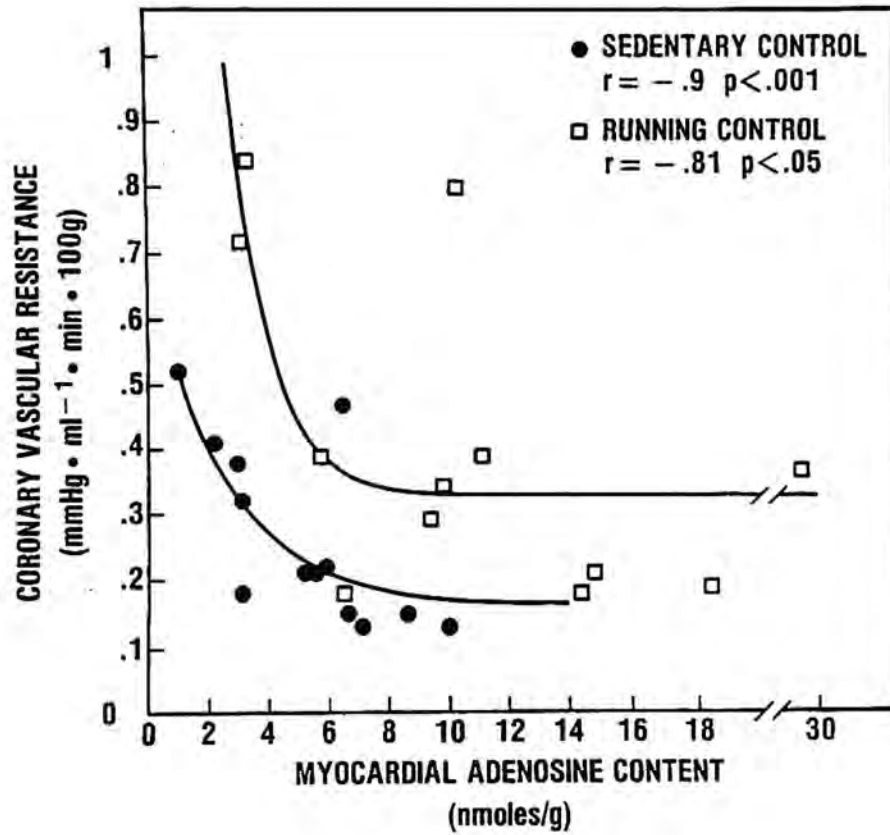
Figure 10. Effects of exercise training on the relationship between myocardial adenosine content and coronary vascular resistance in diabetic rats. Values are plotted for each rat. There is a significant correlation ($r = 0.81$, $p < 0.05$, $n = 12$) between myocardial adenosine content and coronary vascular resistance after a running program.
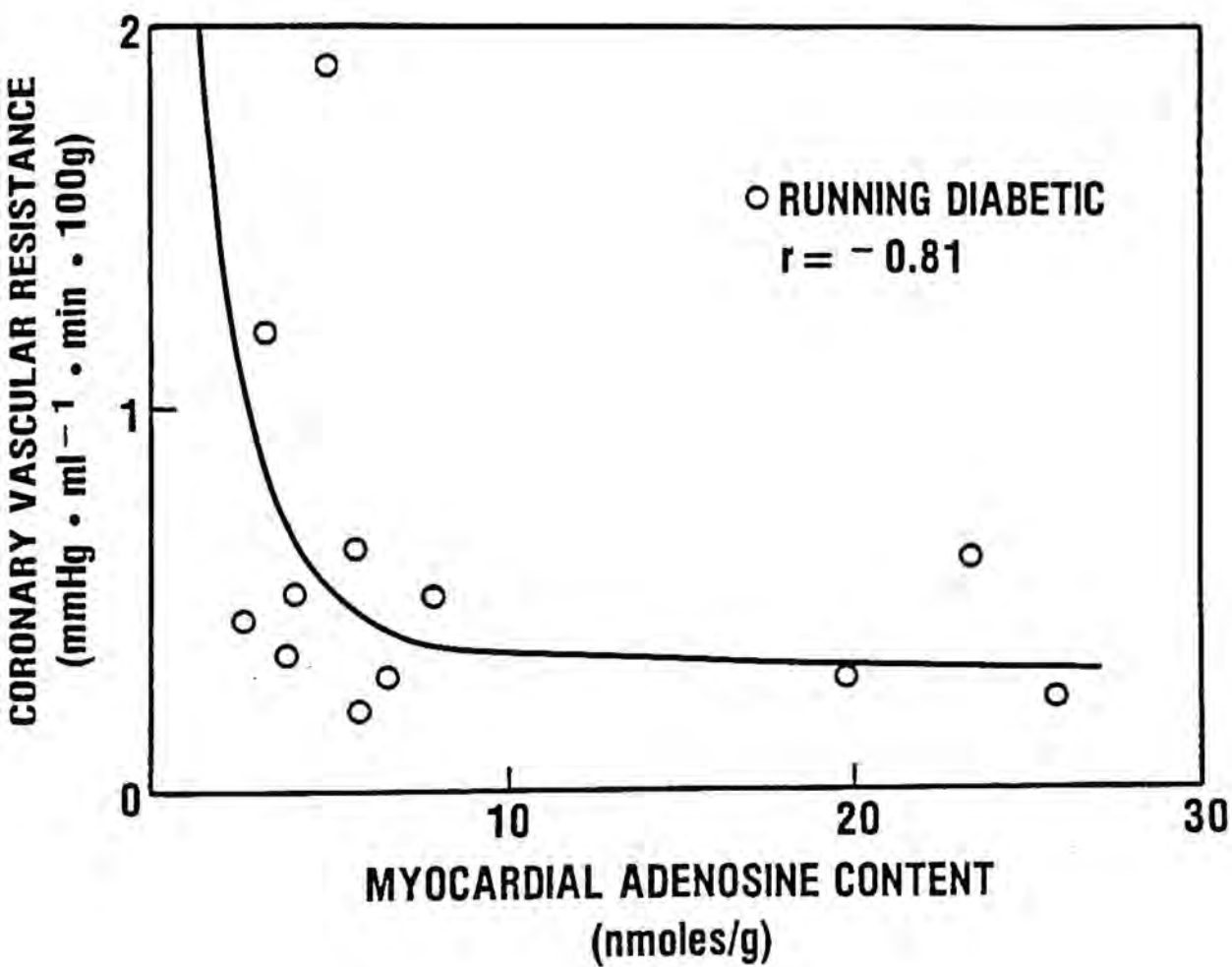
compared to normal dogs both before and after sympathetic stimulation, and heart rate increased significantly in normal and diabetic dogs with sympathetic stimulation. There were no differences in total peripheral resistance (TPR) between any of the treatments although TPR tended to be elevated in the DNE group. Resting cardiac output (CO) was similar in normal and diabetic dogs, and CO increased significantly with sympathetic stimulation in the normal dogs only.

There were no significant differences in either LVP or dPdt between normal and diabetic dogs at rest. Sympathetic stimulation caused 1.7 - fold and 1.6 - fold increases in LVP and 2.8 and 2.6 - fold increases in dPdt in normal and diabetic dogs, respectively. PRP, which is used as an index of cardiac performance, and coronary blood flow (CBF) were elevated in the diabetic dogs as compared to normal dogs at rest and after norepinephrine administration. Sympathetic stimulation caused a two - fold increase in PRP in both normal and diabetic dogs, and 2.4 and 2.8 - fold increases in CBF in normal and diabetic dogs, respectively. CVR was lower in diabetic dogs than normal dogs before and after norepinephrine (Figure 11), and norepinephrine administration caused a greater change (43% for DNE and 37% for NNE) in CVR in diabetic dogs than normal dogs.

b. Blood Data

Table 3 summarizes blood gas data (arterial PO_2, PCO_2, pH, and oxygen concentration [(O_2)], coronary venous pH and (O_2), oxygen extraction and myocardial oxygen consumption [MVO_2] for normal and diabetic dogs at rest and during sympathetic stimulation. There were no significant differences in arterial PO_2 or PCO_2 between any of the

TABLE 2

HEMODYNAMIC DATA

CONTROL STUDY

	NC (N = 10)	NNE (N = 10)	DC (N = 6)	DNE (N = 6)
MABP (mmHg)	114.9 ± 4.7	155.0 ± 7.8*	135.3 ± 3.5†	194.5 ±12.7*
HR (bpm)	136.7 ± 7.3	175.1 ± 9.0*	170.5 ± 11.2†	221.7 ± 7.2*†
TPR (mmHg· min·1^{-1})	51.2 ± 8.3	47.1 ± 4.1	46.5 ± 2.2	77.0 ± 16.7
CO (1·min^{-1})	2.52 ± 0.15	3.69 ± 0.43*	3.11 ± 0.21	4.21 ± 1.5
LVP (mmHg)	132 ± 6.3	225 ± 8.4*	143 ± 2.8	230 ± 16.5*
dP/dt (mmHg/sec^2)	2775 ± 225	7750 ± 754*	3245 ± 421	8500 ± 1016*†
PRP (mmHg·bpm)	18148 ± 1419	39567 ± 2563*	23021 ± 1683†	48303 ± 3207*†
CBF (ml·min^{-1}· 100g^{-1})	53.4 ± 4.3	128.2 ± 20.4*	103.6 ± 24.5†	295.1 ± 77.7*†
CVR (mmHg·ml^{-1}· min·100g)	2.27 ± 0.2	1.44 ± 0.19*	1.56 ± 0.23†	.886 ± 0.22*†

values expressed as mean ± SEM
* $p < 0.05$ (compared to its own control)
† $p < 0.05$ (compared to same treatment in normal group)
NC = Normal Control   DC = Diabetic Control
NNE = Normal w/Norepinephrine   NNE = Diabetic w/Norepinephrine

Figure 11. Effects of diabetes on coronary vascular resistance during basal and norepinephrine - stimulated conditions in the dog. Values are expressed as mean ± SEM for normal (n = 10) and diabetic (n = 6) dogs under control (C) conditions and with norepinephrine infusion (NE). *Denotes significant difference (p < 0.05) compared to its own control. †Denotes significant difference (p < 0.05) compared to same treatment in normal group.
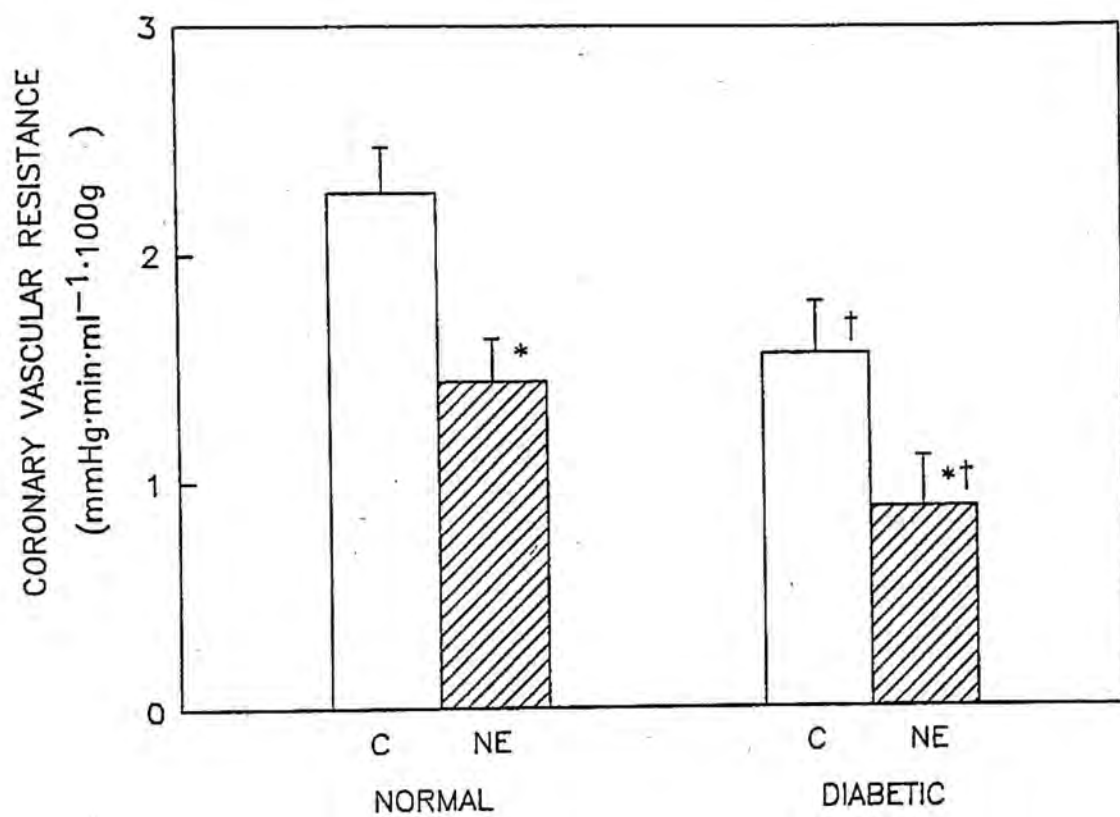
* † p < 0.05

TABLE 3

BLOOD GAS DATA

CONTROL STUDY

	NC (n = 10)	NNE (n = 10)	DC (n = 6)	DNE (n = 6)
Arterial PO_2 (mmHg)	83.6 ± 4.79	66.3 ± 2.28	83.3 ± 10.04	72.8 ± 5.13
Arterial PCO_2 (mmHg)	27.7 ± 1.4	30.7 ±1.5	25.6 ± .88	28.1 ±2.3
Arterial pH	7.434 ± 0.01	7.497 ± 0.02	7.428 ± 0.01	7.326*† ± 0.03
CS pH	7.392 ± 0.01	7.341 ± 0.02	7.383 ± 0.02	7.330 ± 0.07
Arterial (O_2) $(mlO_2/100ml)$	20.32 ± 0.65	22.58 ± 0.95	20.52 ± 0.87	24.18 ± 1.33*
CS (O_2) $(mlO_2/100ml)$	3.84 ± 0.33	7.15 ± 0.76*	3.48 ± 0.26	12.75 ± 1.89*†
A - CS (O_2) $(mlO_2/100ml)$	16.48 ± 0.75	15.43 ± 1.1	17.03 ± 0.95	12.55 ± 1.3*
MVO_2 $(mlO_2 \cdot min \cdot 100g^{-1})$	8.48 ± 1.04	18.28 ± 3.57*	17.29 ± 2.5†	34.28 ± 11.4

values expressed as mean ± SEM
* $p < 0.05$ (compared to its own control)
† $p < 0.05$ (compared to same treatment in normal group)
NC = Normal Control   DC = Diabetic Control
NNE = Normal w/Norepinephrine   DNE = Diabetic w/Norepinephrine

Figure 12.   Effects of diabetes on myocardial oxygen extraction during basal and norepinephrine - stimulated conditions in the dog.   Values are expressed as mean $\pm$ SEM for normal (n = 10) and diabetic (n = 6) groups under control (C) conditions and with norepinephrine infusion (NE).   *Denotes significant difference (p < 0.05) compared to its own control.   †Denotes sigificant difference (p < 0.05) compared to same treatment in normal group.

The y-axis is labeled "MYOCARDIAL OXYGEN EXTRACTION ($mlO2 \cdot 100ml^{-1}$)" with scale marks at 0, 5, 10, 15, 20. The x-axis shows groups: C, NE under NORMAL, and C, NE under DIABETIC. The NE bar under DIABETIC is marked with an asterisk (*).

* p < 0.05

treatment groups, and there were no differences in arterial pH between normal and diabetic dogs at rest. Norepinephrine administration caused a significant decrease in arterial pH in the diabetic dogs only. Coronary sinus pH was similar between all groups, and coronary sinus oxygen concentration was similar between normal and diabetic dogs at rest. Sympathetic stimulation caused increases in coronary sinus (O_2) of 186% and 266% in normal and diabetic dogs, respectively, and the increase in coronary sinus oxygen concentration in diabetic dogs was significantly higher than the coronary sinus oxygen increase in normal dogs. The large increase in coronary sinus (O_2) in the DNE groups caused a significant decrease in myocardial oxygen extraction, as shown in Fig. 12. Myocardial oxygen consumption was elevated at rest in the diabetic dogs compared to normal dogs, and sympathetic stimulation resulted in a significant increase in MVO_2 in normal dogs, only, although the mean MVO_2 doubled in both normal and diabetic dogs.

    c. <u>Metabolic data</u>

    Blood glucose, lactate and pyruvate data are shown in Table 4. There were no significant differences in arterial or coronary sinus lactate and pyruvate concentrations and lactate/pyruvate (L/P) ratios between any of the treatment groups. Diabetic arterial and coronary sinus glucose concentrations and glucose uptake were significantly greater than those of normal dogs both at rest and with norepinephrine - stimulation. Glucose uptake did not change significantly with sympathetic stimulation in either normal or diabetic dogs. Glycosylated hemoglobin, expressed as a percentage of total hemoblobin, ranged from 6 - 8% in normal dogs, and 14 - 19% in diabetic dogs.

TABLE 4

METABOLIC DATA

CONTROL STUDY

	NC (n = 10)	NNE (n = 10)	DC (n = 6)	DNE (n = 6)
Arterial Lactate(mmol/l)	1.99 ± 0.5	1.61 ± 0.32	0.91 ± 0.25	1.49 ± 0.41
CS Lactate (mmol/l)	1.32 ± 0.28	1.28 ± 0.26	0.74 ± 0.25	1.45 ± 0.43
Arterial Pyruvate (mmol/l)	0.077 ± 0.012	0.057 ± 0.012	0.048 ± 0.013	0.064 ± 0.013
CS Pyruvate (mmol/l)	0.061 ± 0.01	0.064 ± 0.01	0.068 ± 0.004	0.101 ± 0.01
CS L/P Ratio	17.93 ± 5.8	16.93 ± 1.0	11.18 ± 3.57	14.4 ± 5.1
Arterial Glucose (mg/100ml)	87 ± 2.6 ± 21.3	103 ± 6.4	259 ± 23.4†	341 ± 21.3†
CS Glucose (mg/100ml)	78 ± 5.7	89 ± 4.4	227 ± 36.0†	302 ± 29.0†
Glucose Uptake (g·min^{-1}·100g^{-1})	0.54 ± 0.44	1.57 ± 0.61	4.2 ± 1.6†	9.3 ± 3.9†

values expressed as mean ± SEM
* $p < 0.05$ (compared to its own control)
† $p < 0.05$ (compared to same treatment in normal group)
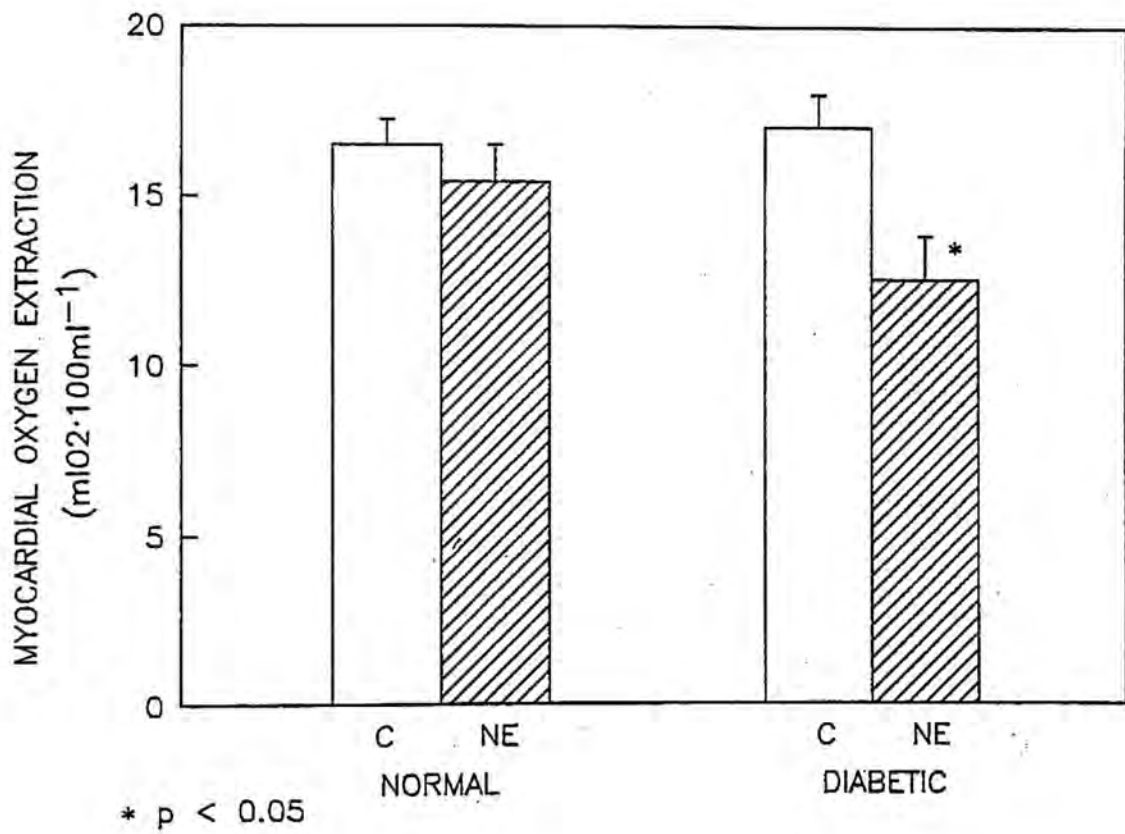NC = Normal Control   DC = Diabetic Control
NNE = Normal w/Norepinephrine   DNE = Diabetic w/Norepinephrine

d.   Adenosine data

Table 5 contains arterial and coronary sinus plasma adenosine concentrations, changes in adenosine concentration across the myocardium, and adenosine release/uptake (ADOR) data. Where values for ADOR are greater than zero, adenosine release is indicated, and where values are less than zero, adenosine uptake is indicated. As shown in Figures 13a and 13b, there were no significant differences in arterial adenosine concentration between NC, NNE, DC, and DNE treatment groups, although arterial adenosine tended to be elevated in the DNE group.   Coronary sinus adenosine concentration was significantly elevated in the diabetic group at rest compared to the normal group at rest, and increased significantly in the normal dogs with sympathetic stimulation, but not in the diabetic dogs. The change in adenosine concentration across the myocardium, expressed as the difference between coronary sinus and arterial adenosine concentration, increased in normal dogs with sympathetic stimulation due to the increase in coronary sinus adenosine. As demonstrated in Figure 14, there was a 16 - fold increase in ADOR with norepinephrine infusion in the normal dogs. The increase in arterial adenosine and decrease in coronary sinus adenosine in diabetic dogs with norepinephrine infusion led to an increase in adenosine extraction, but the changes were not significant.

e.   The Relationship between cardiac performance and myocardial oxygen consumption

The effects of sympathetic stimulation on cardiac performance, as expressed by PRP, and myocardial oxygen consumption are represented in Figures 15a and 15b. The top panel (15a) shows a significant linear correlation between PRP and MVO_2 in normal dogs ($r = 0.72$, $p < 0.05$).

TABLE 5

ADENOSINE DATA

CONTROL STUDY

	NC (N = 10)	NNE (N = 10)	DC (N = 6)	DNE (N = 6)
Arterial Adenosine (pmoles/ml)	143.0 ± 37.3	203.6 ± 59.8	194.8 ± 50.4	516.8 ± 281
CS Adenosine (pmoles/ml)	188.5 ± 47.6	696.9 ± 201.0*	471.3 ± 156.0†	388.1 ± 101.0
CS - Arterial Adenosine (pmoles/ml)	44.2 ± 44.9	492.4 ± 199.8*	276.5 ± 156.0	-213.8 ± 28.5
Adenosine Release/uptake (nmoles·min^{-1}· 100g^{-1})	3.9 ± 2.6	65.0 ± 27.5*	11.42 ± 8.3	-39.6 ± 65.0

values expressed as mean ± SEM
* $p < 0.05$ (compared to its own control)
† $p < 0.05$ (compared to same treatment in normal group)
NC = Normal Control   DC = Diabetic Control
NNE = Normal w/Norepinephrine   DNE = Diabetic w/Norepinephrine

Figure 13. Effects of diabetes on arterial, coronary sinus, and (coronary
sinus - arterial) adenosine concentrations during basal and norepinephrine -
stimulated conditions in the dog. Values are expressed as mean ± SEM for
normal (a, n = 10) and diabetic (b, n = 6) dogs under control conditions (C)
and with norepinephrine infusion (NE). *Denotes significant difference
(p < 0.05) compared to its own control.   †Denotes significant difference
(p < 0.05) compared to same treatment in control group.

NORMAL DOGS
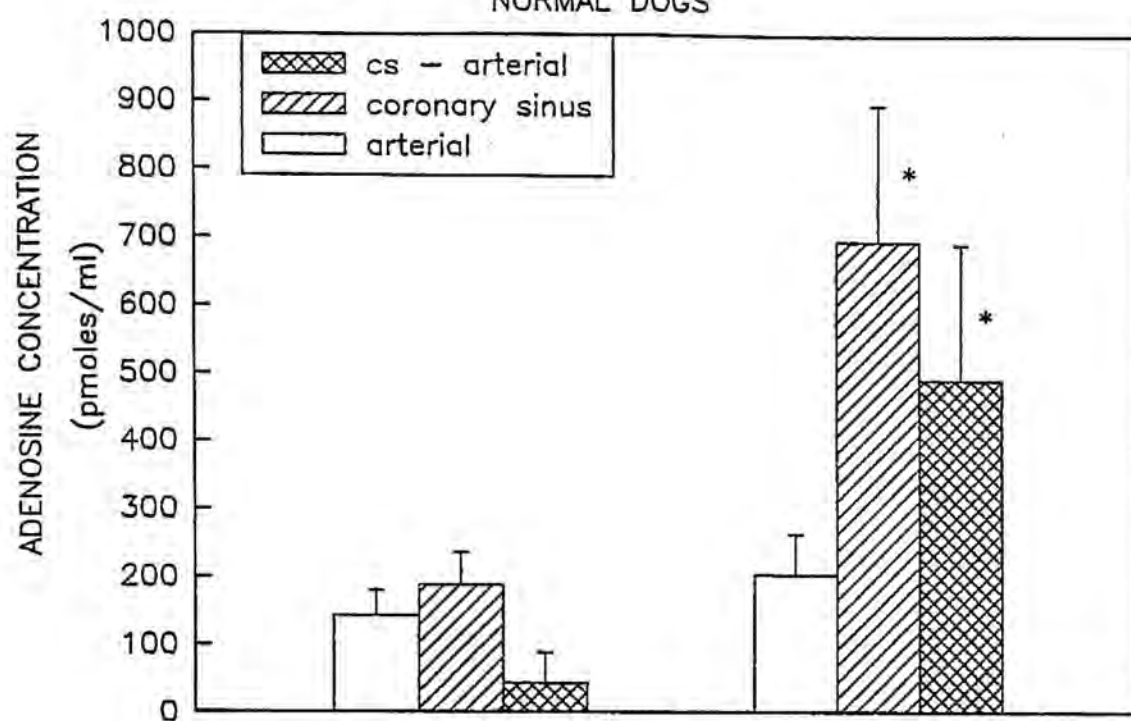
DIABETIC DOGS
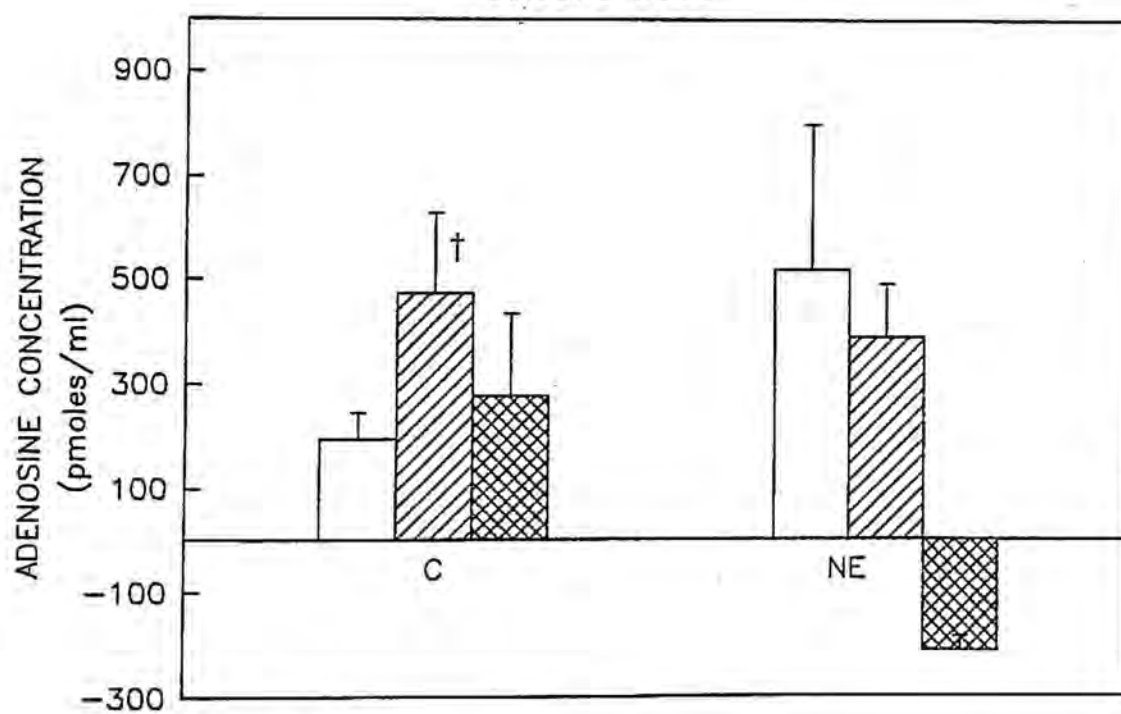
* † p < 0.05

Figure 14. Effects of diabetes on myocardial adenosine release/uptake during basal and norepinephrine - stimulated conditions in the dog. Values are expressed as mean ± SEM for normal (n = 10) and diabetic (n = 6) groups under control (C) conditions and with norepinephrine infusion (NE). *Denotes significant difference (p < 0.05) compared to its own control.
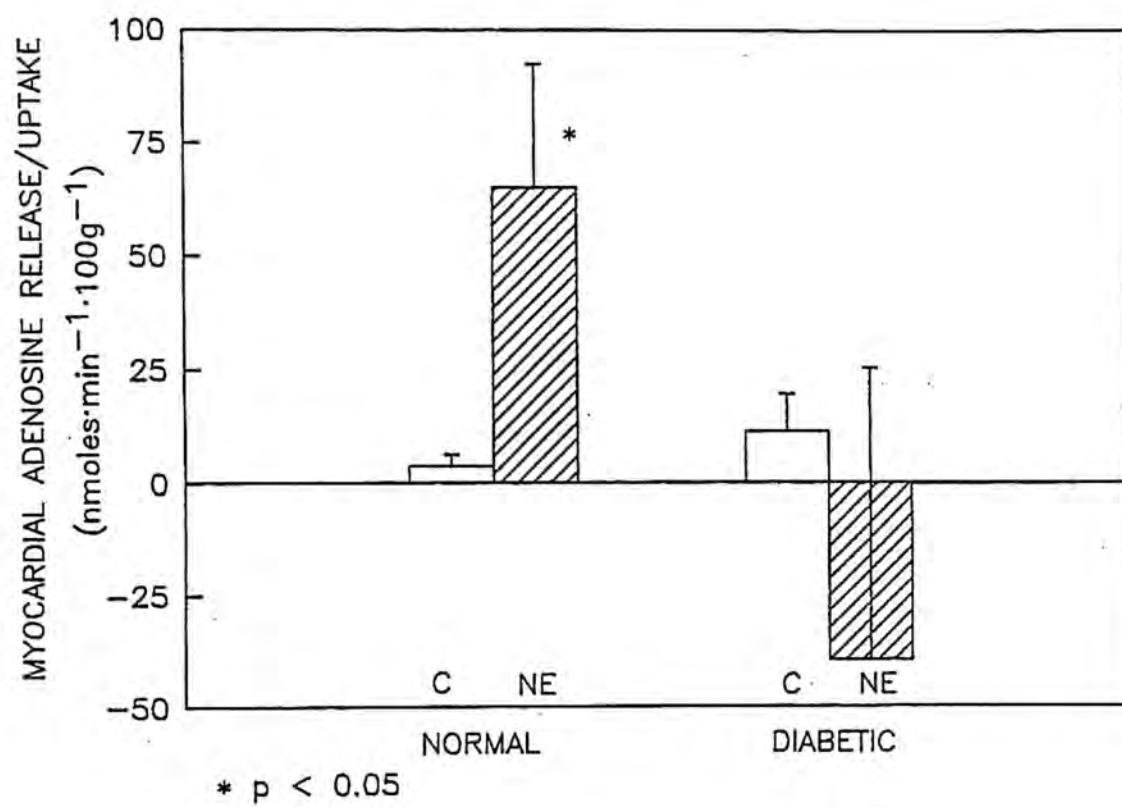
* p < 0.05

Both resting and norepinephrine - stimulated values are expressed. The bottom panel (15b) shows the relationship between PRP and MVO_2 in diabetic dogs. In this case there is not a significant correlation between the two parameters ($r = 0.41$, $p > 0.05$). This figure demonstrates that increases in cardiac performance, as represented by PRP, are associated with proportional increases in oxygen consumption in normal dogs, but not in diabetic dogs.

  f. The relationship between cardiac contractility and myocardial oxygen consumption

Figure 16 represents the relationship between contractility, as expressed by dPdt, and MVO_2 at rest and during sympathetic stimulation in normal and diabetic dogs. The linear regression coefficients for normal and diabetic groups are 0.56 and 0.73, respectively, and both of these correlations are significant. This figure shows that oxygen consumption increases to a greater extent with increased contractility in diabetic dogs than normal dogs. In addition, when compared to Figure 14b, oxygen consumption in the diabetic dogs correlates more closely with changes in contractility than with changes in pressure - rate work.

  g. The relationship between myocardial oxygen consumption and coronary blood flow

The linear relationship between myocardial oxygen consumption and coronary blood flow with sympathetically - mediated increases in cardiac work is shown in Figures 17a and 17b. Normal dogs are represented on the top panel (17a) and diabetic dogs are represented on the bottom (17b). There are similar significant correlations betwen MVO_2 and CBF for normal ($r = 0.89$, $p < 0.05$) and diabetic ($r = 0.94$, $p < 0.05$) groups. This

Figure 15. Relationship between cardiac performance and myocardial oxygen consumption in normal and diabetic dogs during basal and norepinephrine - stimulated conditions. Values are plotted for each dog. Cardiac performance, expressed as left ventricular pressure X heart rate, correlated significantly ($r = 0.72$, $p < 0.05$, $n = 10$) with myocardial oxygen consumption in normal dogs (a) but not in diabetic dogs (b), ($r = 0.41$, $p > 0.05$, $n = 6$).

NORMAL DOGS

DIABETIC DOGS

Figure 16. Relationship between cardiac contractility and myocardial oxygen consumption in normal and diabetic dogs during basal and norepinephrine - stimulated conditions. Values are plotted for each dog in normal (open circles) and diabetic (filled circles) group. Myocardial contractility, represented by dP/dt, is more highly correlated with myocardial oxygen consumption in the diabetic dogs ($r = 0.73$, $p < 0.05$, $n = 6$) than in the normal dogs ($r = 0.56$, $p < 0.05$, $n = 10$).
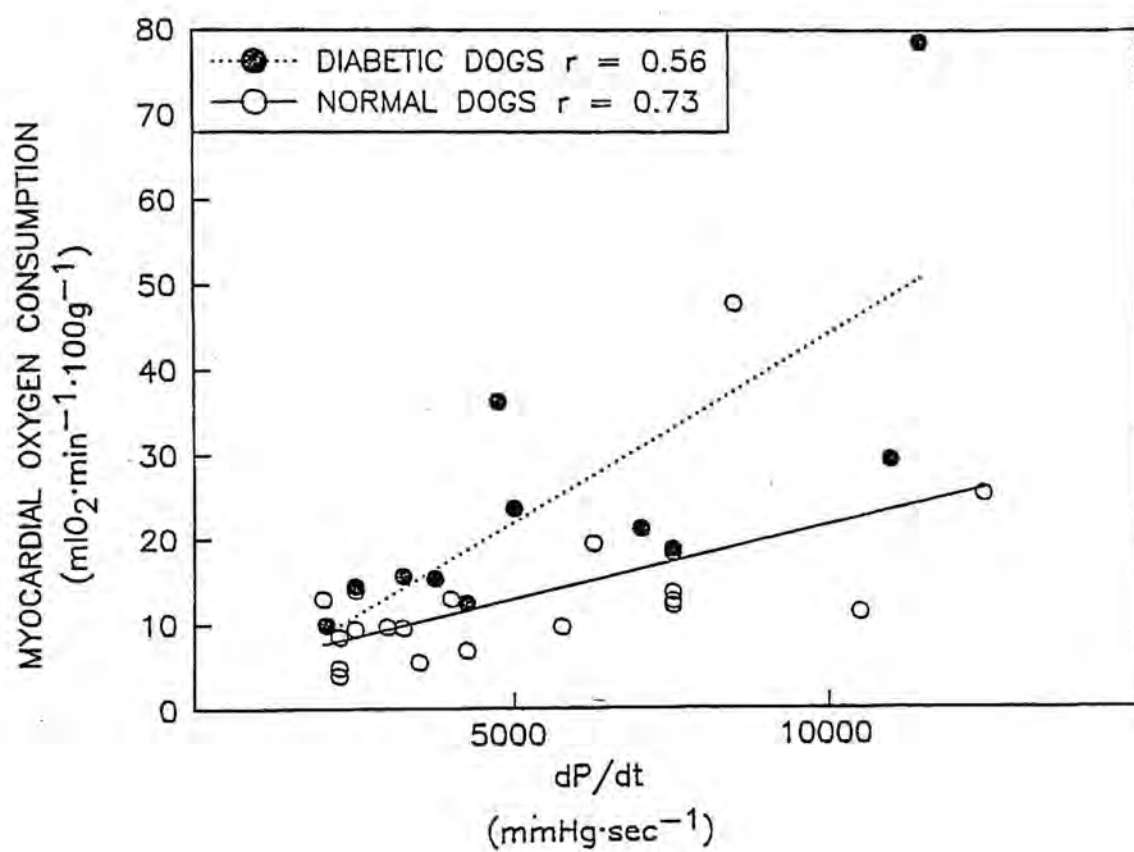
Figure 17. Relationship between myocardial oxygen consumption and coronary blood flow in normal and diabetic dogs during basal and norepinephrine - stimulated conditions. Values are expressed for each dog. Myocardial oxygen consumption correlated with coronary blood flow in both normal (a) ($r = 0.89$, $p < 0.05$, $n = 10$) and diabetic (b) ($r = 0.88$, $p < 0.05$, $n = 6$) groups.

NORMAL DOGS
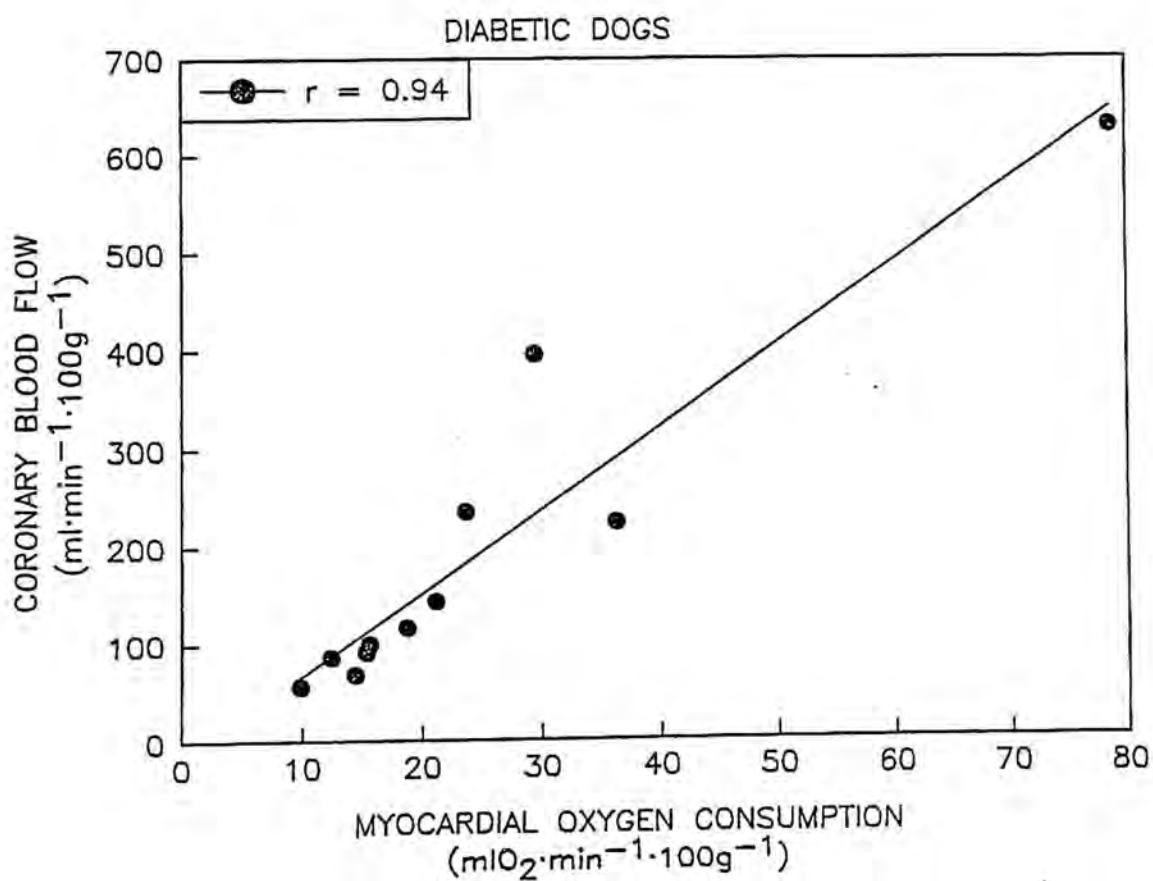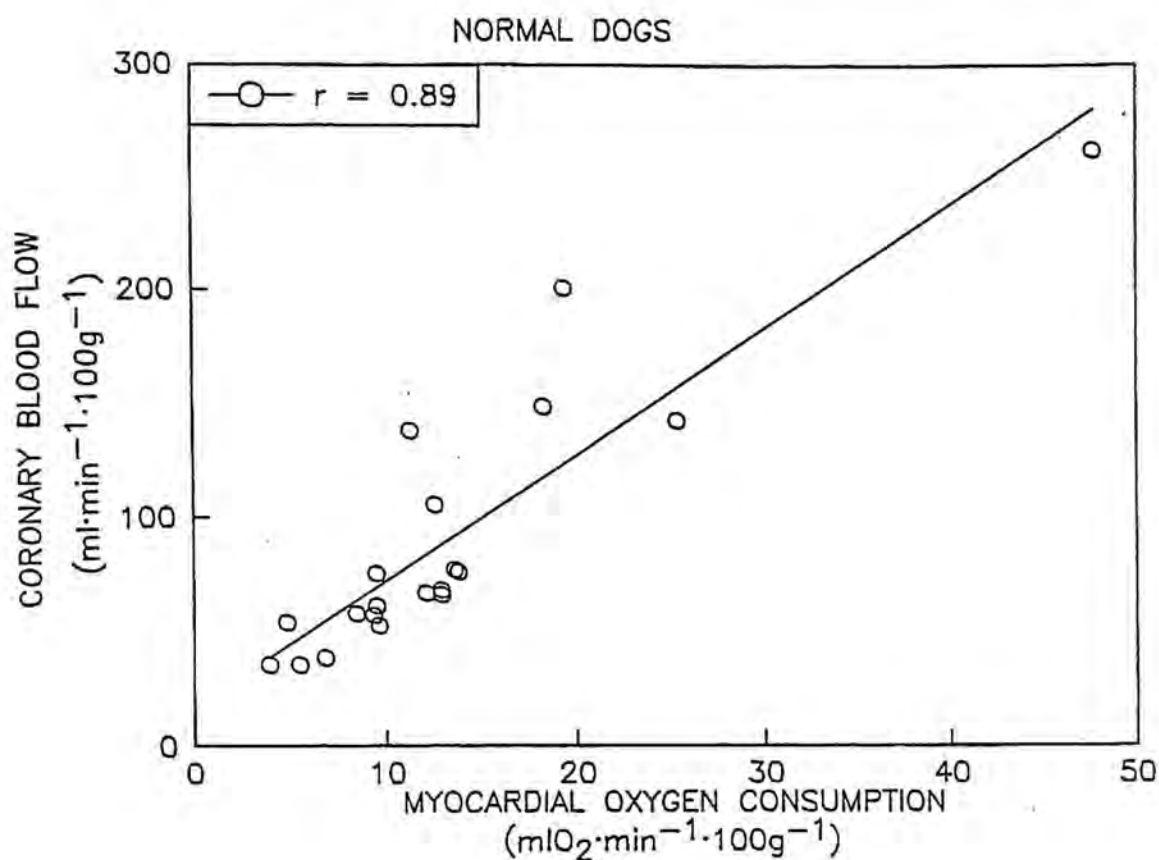
DIABETIC DOGS

figure illustrates that CBF increases with norepinephrine - induced increases in MVO_2 in diabetic dogs as well as in normal dogs.

  h. The relationship between cardiac contractility and coronary
     blood flow

In Fig. 18, the linear relationship between dPdt and CBF is shown for normal and diabetic dogs. The correlation coefficient for normal and diabetic regression lines are 0.65 and 0.83, respectively. Both correlations are significant. These data show that as seen in the relationship between contractility and oxygen consumption, coronary blood flow correlates more closely with contractility in the diabetic dogs than in normal dogs.

  i. The relationship between cardiac performance and coronary
     vascular resistance

The linear correlation between PRP and CVR is demonstrated in Figures 19a and 19b. Normal dogs are represented above (19a), and diabetic dogs are represented below (19b). CVR correlates significantly with PRP in the normal dogs ($r = 0.58$, $p < 0.05$) but does not correlate with PRP in the diabetic dogs ($r = 0.48$, $p > 0.05$). This figure indicates that increases in CVR observed in the diabetic dogs with sympathetic stimulation may have been mediated more strongly by factors other than increases in cardiac work as measured by PRP.

  j. The relationship between cardiac contractility and coronary
     vascular resistance

The effects of sympathetic stimulation on dP/dt and CVR are illustrated in Fig. 20, for normal and diabetic dogs. There are significant correlations between dPdt and CVR in both groups. However, the regression correlation coefficient is higher in the diabetic dogs

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Figure 18. Relationship between cardiac contractility and coronary blood flow in normal and diabetic dogs during basal and norepinephrine - stimulated conditions. Values are expressed for each dog in normal (open circles, solid line) and diabetic (filled circles, dotted line) groups. Contractility, represented by dP/dt, is better correlated with coronary blood flow in the diabetic dogs (r = 0.83, p < 0.05, n = 6) than in the normal dogs (r = 0.65, p < 0.05, n = 10).
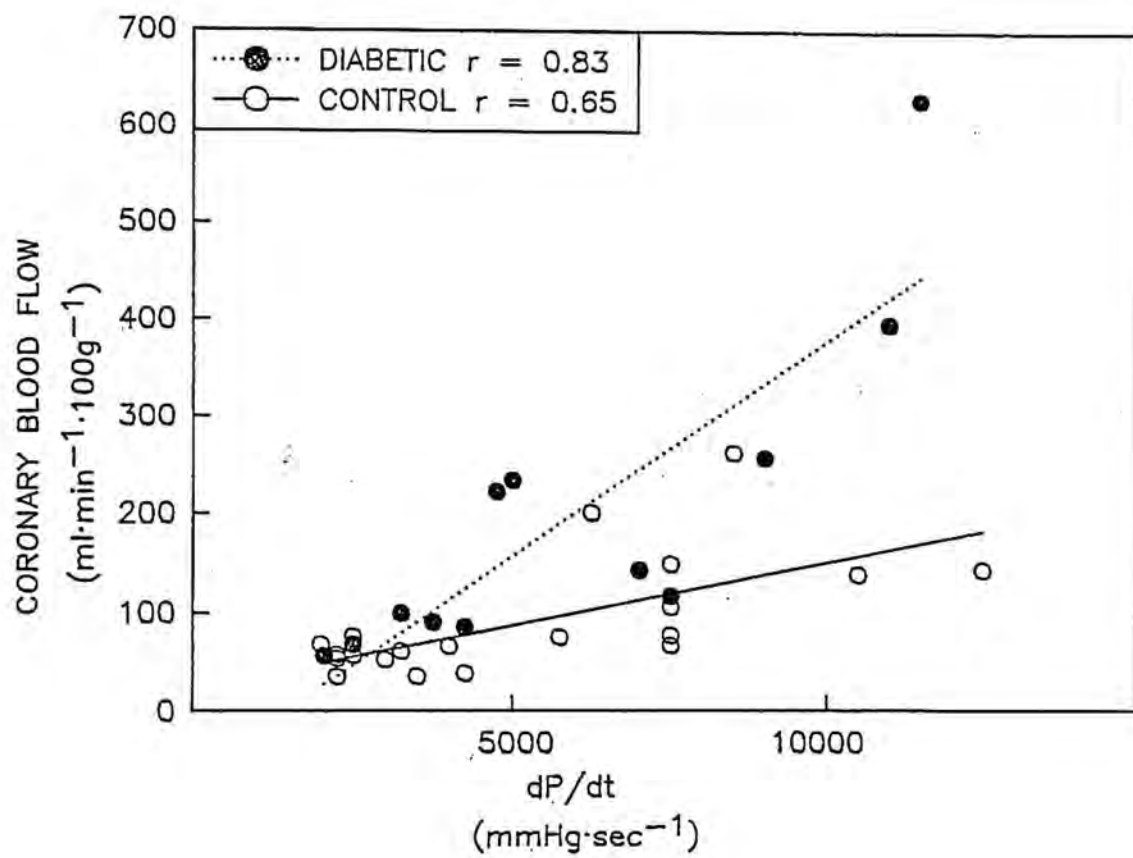
Figure 19. Relationship between cardiac performance and coronary vascular resistance in normal (a) and diabetic (b) dogs during basal and norepinephrine - stimulated conditions. Values are expressed for each dog. Cardiac performance, represented by left ventricular pressure X heart rate, is correlated with coronary vascular resistance in the normal dogs ($r = 0.58$, $p < 0.05$, $n = 10$) but not in the diabetic dogs ($r = 0.48$, $p > 0.05$, $n = 6$).

NORMAL DOGS

CORONARY VASCULAR RESISTANCE $(mmHg \cdot min \cdot ml^{-1} \cdot 100g^{-})$

LEFT VENTRICULAR PRESSURE X HEART RATE $(mmHg \cdot beats \cdot min^{-1})$

$r = 0.58$

DIABETIC DOGS

CORONARY VASCULAR RESISTANCE $(mmHg \cdot min \cdot ml^{-1} \cdot 100g^{})$

LEFT VENTRICULAR PRESSURE X HEART RATE $(mmHg \cdot beats \cdot min^{-1})$

$r = 0.48$
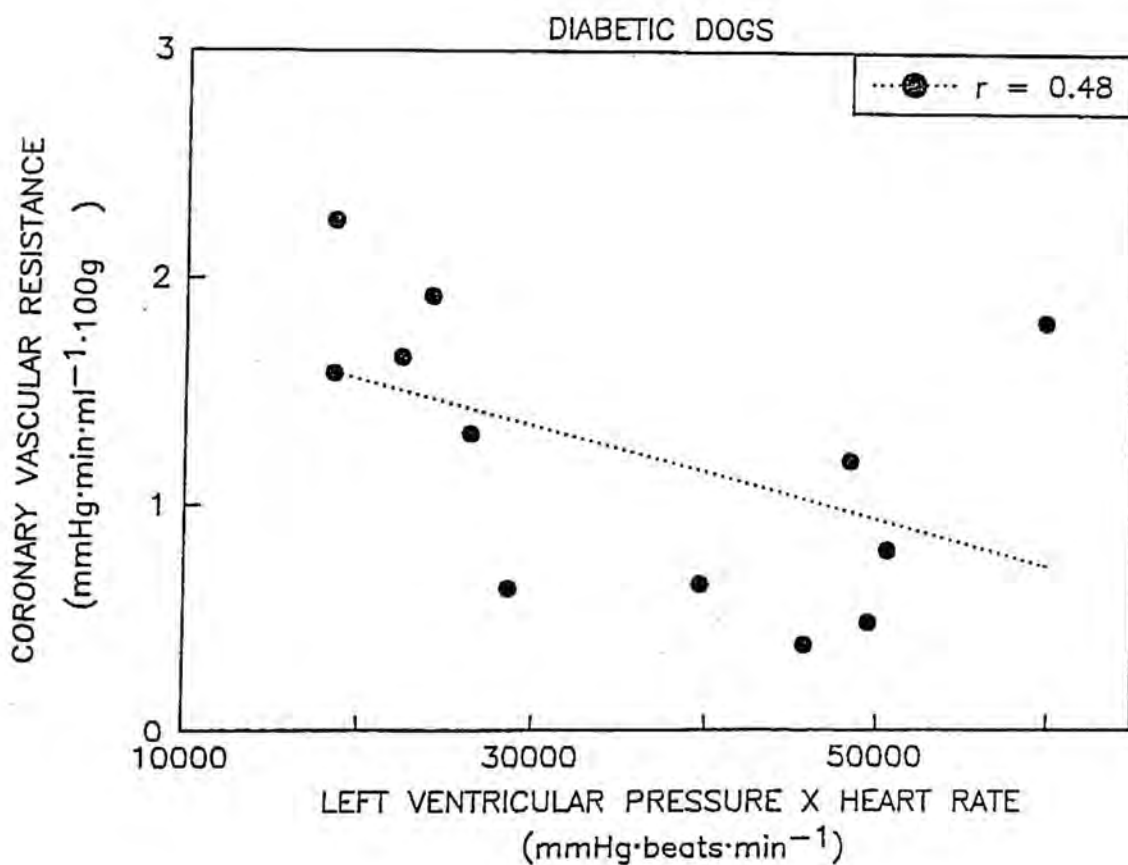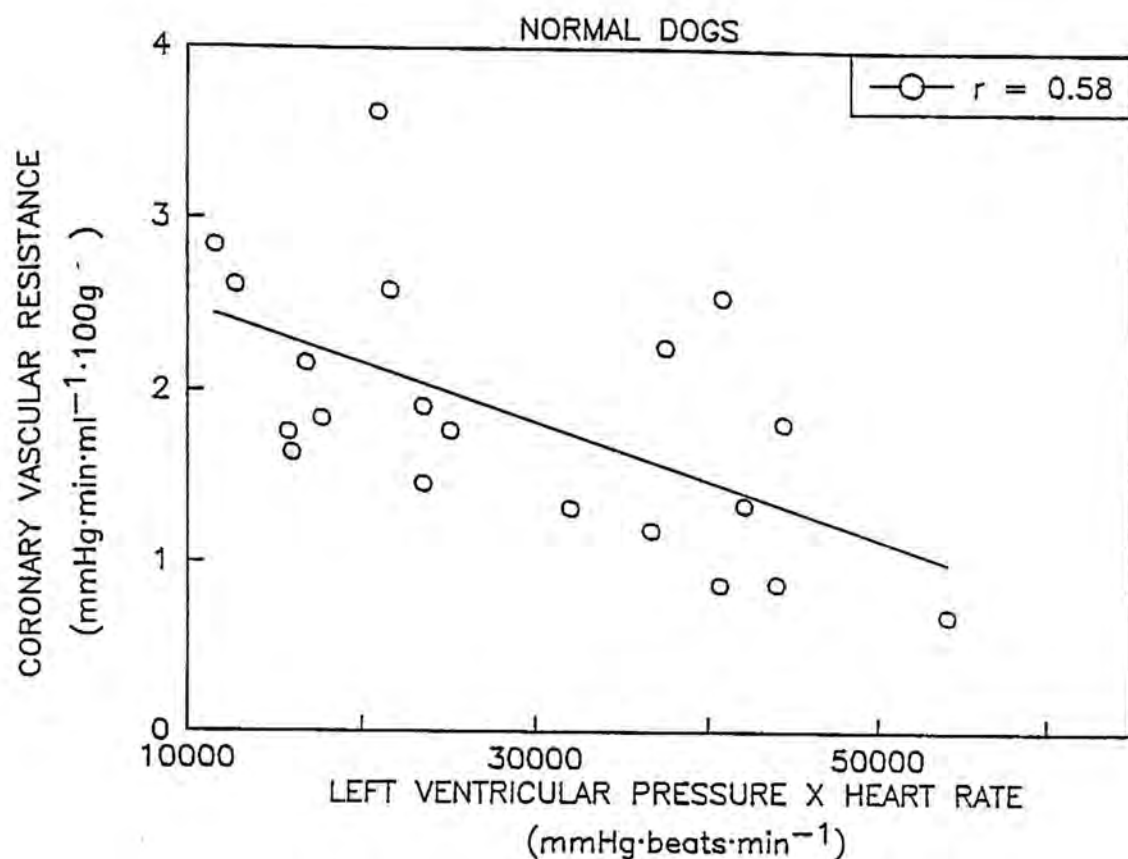
($r = 0.77$, $p < 0.05$) than in the normal dogs ($r = 0.55$, $p < 0.05$).
Analysis of covariance shows the slopes of the lines to be the same,
while the Y - intercept is significantly different, which demonstrates
that at a given dP/dt, CVR is lower in the diabetic group. When compared
with the previous figure, these graphs show that changes in CVR with
sympathetic stimulation are more closely correlated with dP/dt than with
PRP in diabetic dogs.

k. The relationships between cardiac performance, myocardial
   oxygen consumption and myocardial adenosine release/uptake

Figures 21a and 21b demonstrate the linear correlation between
cardiac performance, as expressed by PRP, and myocardial adenosine
release/uptake. Normal dog coordinates are plotted above (21a) and
diabetic dog coordinates are plotted below (21b). ADOR correlates
significantly with PRP in the normal group ($r = 0.5$, $p < 0.05$). However
there is not a significant correlation between ADOR and PRP in the
diabetic group ($r = 0.07$, $p > 0.05$). In Figures 22a and 22b, ADOR is
plotted against increases in MVO_2 for normal dogs (above) and diabetic
dogs (below). Again, there is a significant linear correlation between
MVO_2 and ADOR in the normal dogs ($r = 0.5$, $p < 0.05$), but not in the
diabetic dogs ($r = 0.08$, $p > 0.05$). Therefore, two parameters closely
linked to changes in adenosine release in normal animals, cardiac work
and oxygen consumption, are not associated with adenosine release with
sympathetic stimulation in diabetic dogs.

l. The relationship between myocardial adenosine release/uptake
   and coronary vascular resistance

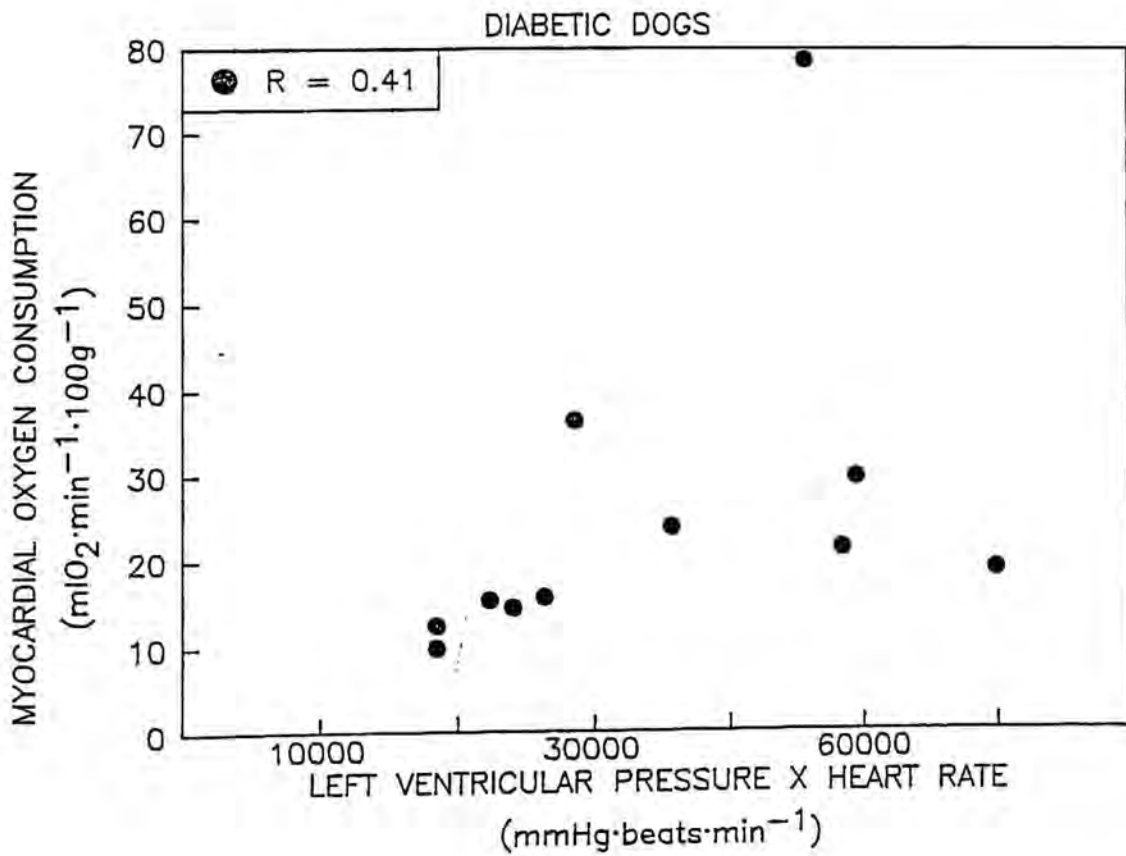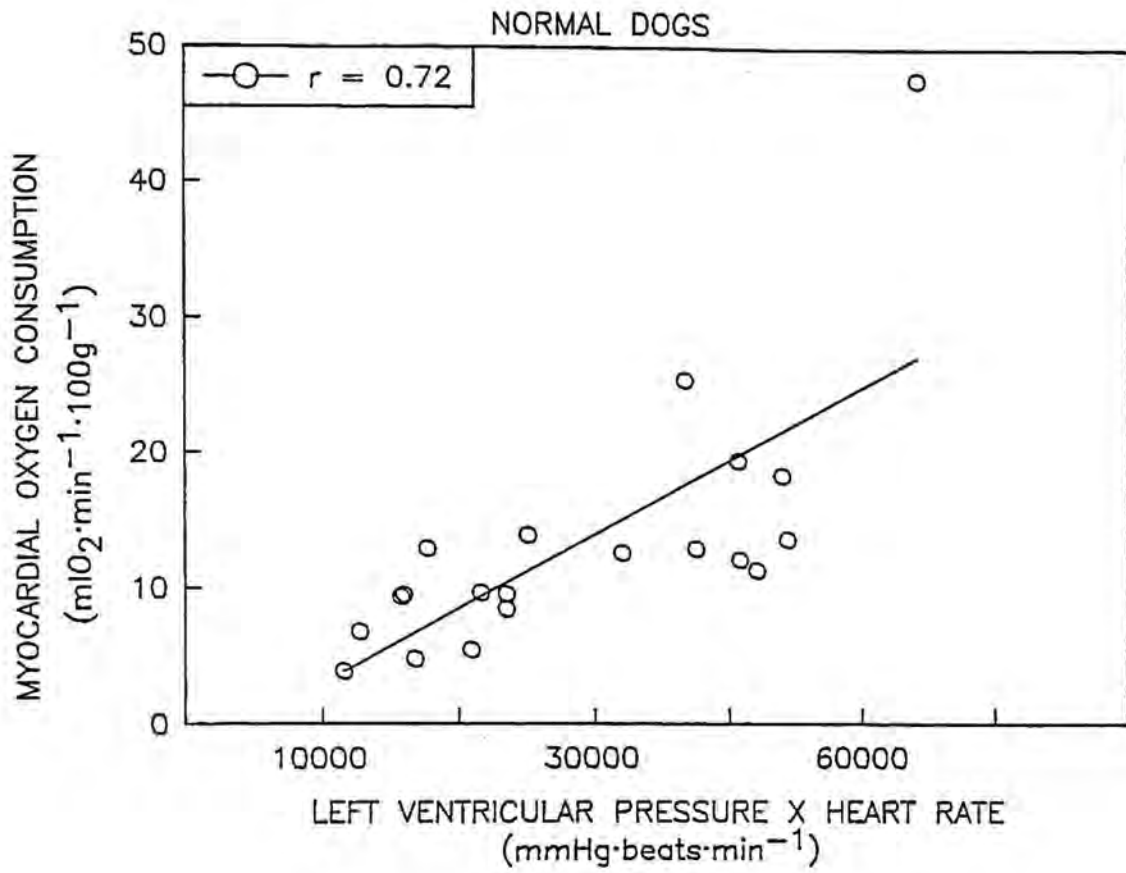The relationships between myocardial adenosine release/uptake and

Figure 20. Relationship between cardiac contractility and coronary vascular resistance in normal and diabetic dogs during basal and norepinephrine – stimulated conditions. Values are expressed for each dog in normal (open circles, solid line) and diabetic (filled circle, dotted line) groups. Contractility, represented by dP/dt, is better correlated with coronary vascular resistance in the diabetic dogs ($r = 0.77$, $p < 0.05$, $n = 6$) than in the normal dogs ($r = 0.55$, $p < 0.05$, $n = 10$).

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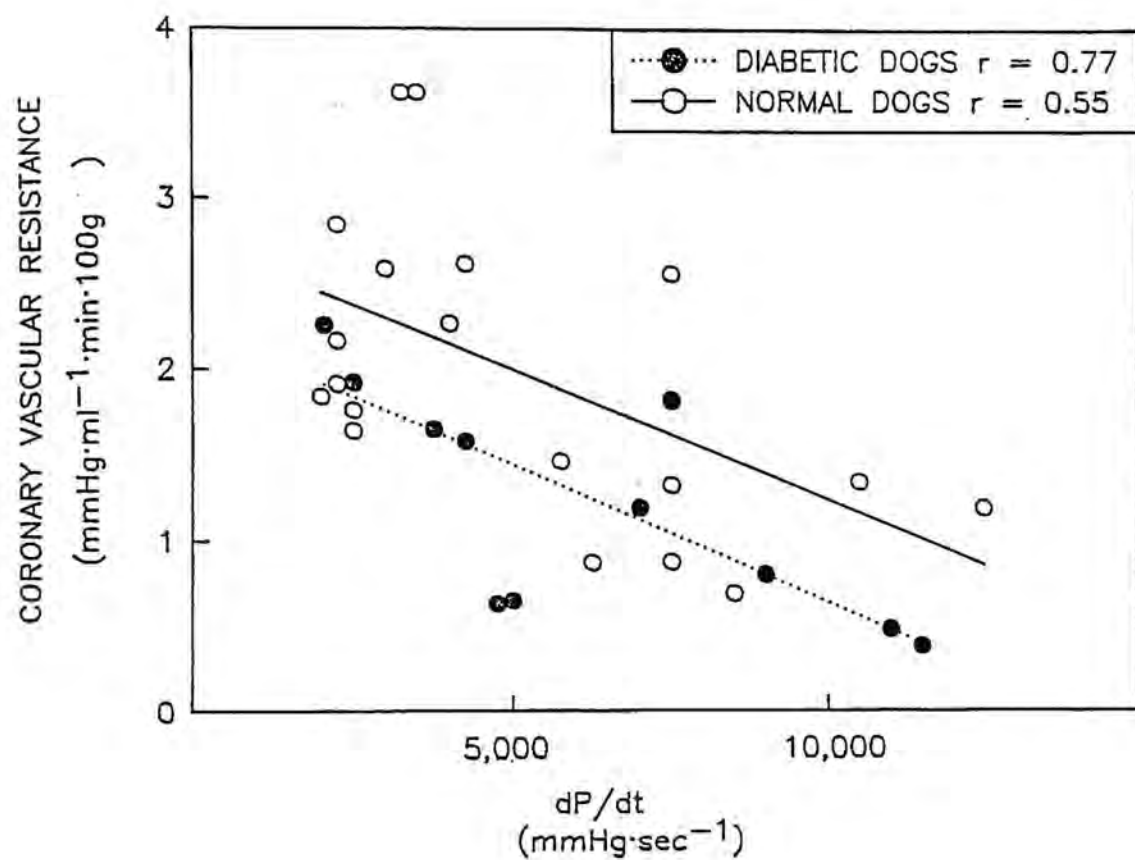
Figure 21. Relationship between cardiac performance and myocardial adenosine release/uptake in normal and diabetic dogs during basal and norepinephrine - stimulated conditions. Values are expressed for each dog for normal (a) and diabetic (b) groups. Cardiac work, represented by left ventricular pressure X heart rate, is significantly correlated with myocardial adenosine release in normal dogs ($r = 0.5$, $p < 0.05$, $n = 10$), but not in diabetic dogs ($r = 0.07$, $p > 0.05$, $n = 6$).

NORMAL DOGS
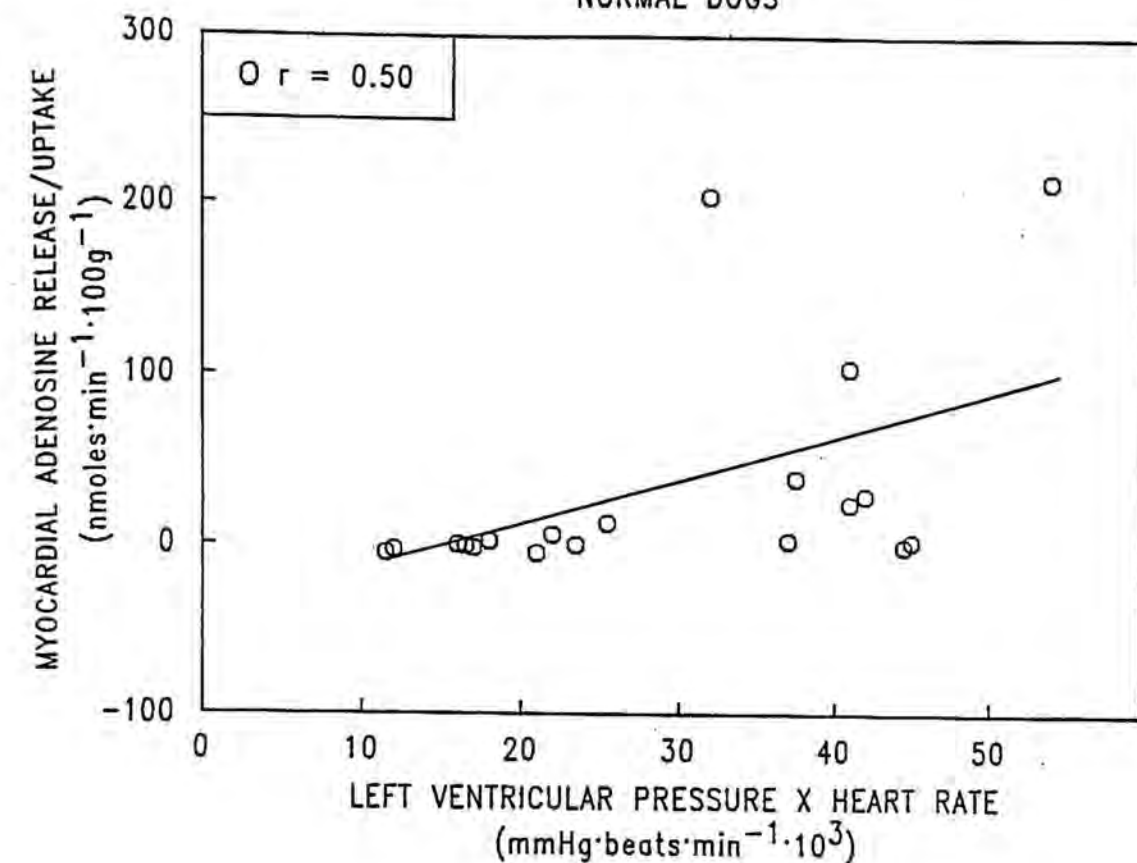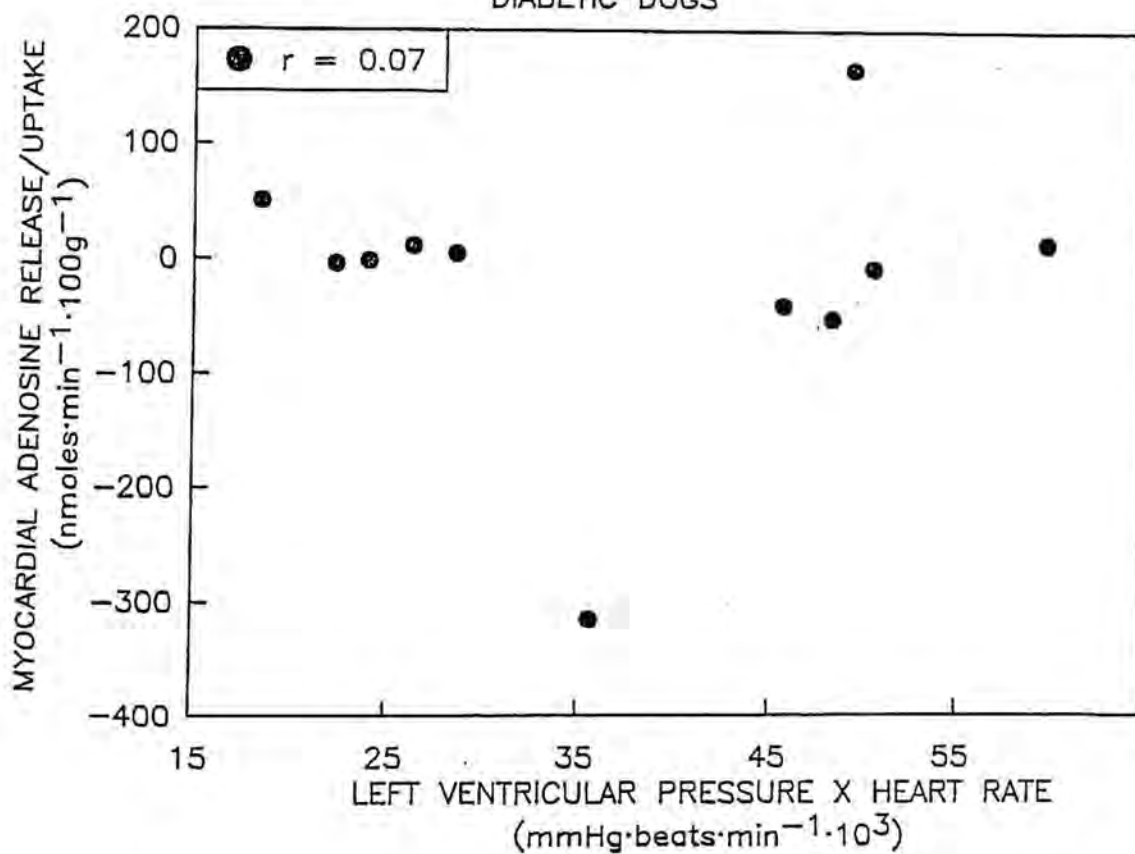
DIABETIC DOGS

Figure 22. Relationship between myocardial oxygen consumption and myocardial adenosine release/uptake in normal and diabetic dogs during basal and norepinephrine - stimulated conditions. Values are expressed for each dog for normal (a) and diabetic (b) groups. Myocardial oxygen consumption correlated with myocardial adenosine release in the normal dogs ($r = 0.5$, $p < 0.05$, $n = 10$) but not in the diabetic dogs ($r = 0.08$, $p > 0.05$, $n = 6$).
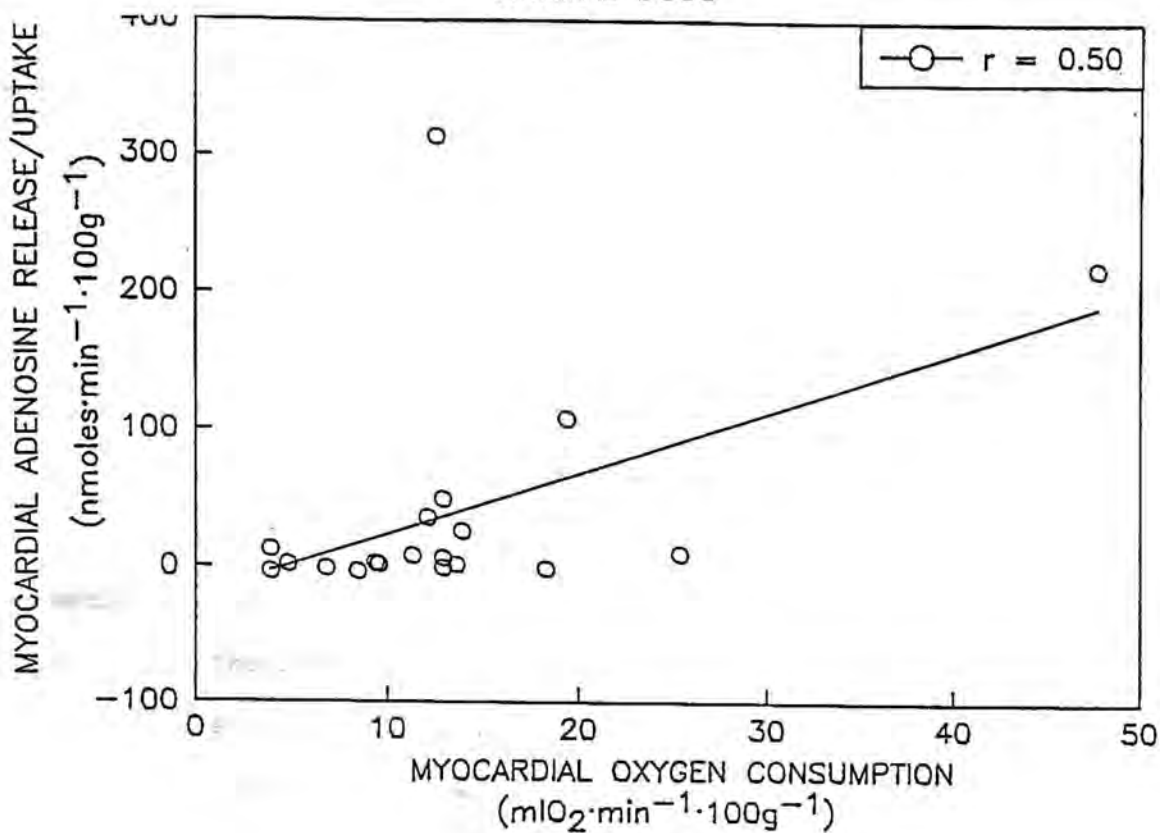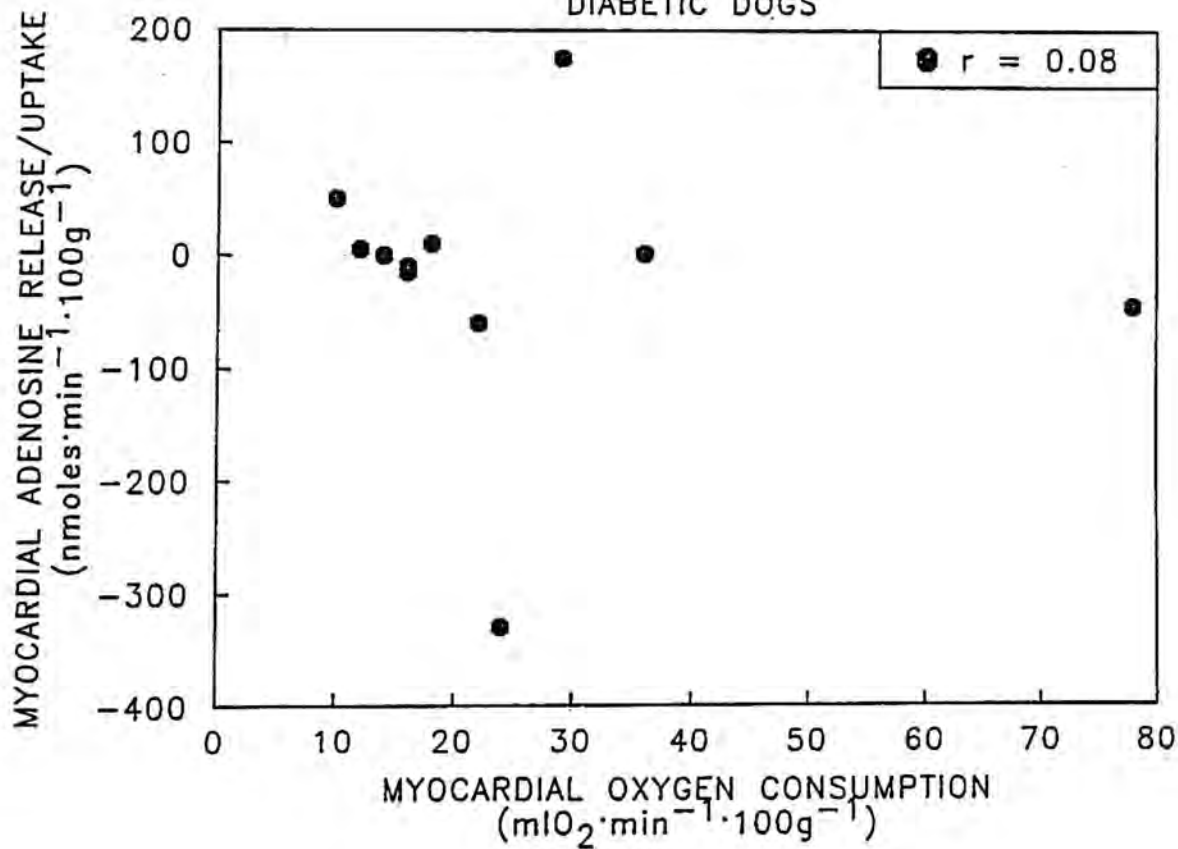
coronary vascular resistance at rest and during sympathetic stimulation for normal and diabetic dogs are shown in Figures 23a and 23b. As shown in the top panel, there is a significant negative curvilinear relationship between CVR and ADOR in the normal dogs ($r = 0.55$, $p < 0.05$). As shown in the bottom panel, there is no significant correlation between the two parameters in the diabetic dogs with norepinephrine - stimulated increases in cardiac work ($r = 0.0$, $p > 0.05$).

To summarize results of the Control Study, both normal and diabetic dogs show increases in CBF and decreases in CVR with sympathetic stimulation. The changes in CBF and CVR are more closely correlated to changes in PRP in normal dogs and to changes in dPdt in diabetic dogs. In normal dogs, ADOR increases with sympathetic stimulation and is significantly correlated with increases in PRP and MVO_2. In diabetic dogs, ADOR does not increase with sympathetic stimulation, and is not correlated with changes in PRP or MVO_2. Finally, the significant negative correlation between ADOR and CVR observed in normal dogs is not present in diabetic dogs.

2. Alpha - adrenergic Blockade Study

    a. Hemodynamic Data

        1. Normal dogs

The effects of intracoronary injections of prazosin, an $alpha_1$ - adrenergic blocker, on hemodynamic parameters during control and norepinephrine - stimulated conditions for normal dogs are shown in Table 6A. In this table and the following tables illustrating normal dog data, normal control (NC) and normal norepinephrine infusion (NNE) refer to basal and stimulated states, respectively, before intracoronary prazosin

Figure 23. Relationship between myocardial adenosine release/uptake and coronary vascular resistance in normal and diabetic dogs during basal and norepinephrine - stimulated conditions. Values are plotted for each dog for normal (a) and diabetic (b) groups. Myocardial adenosine release correlated with coronary vascular resistance in the normal dogs ($r = 0.55$, $p < 0.05$, $n = 10$) but not in the diabetic dogs ($r = 0.0$, $p > 0.05$, $n = 6$).

NORMAL DOGS
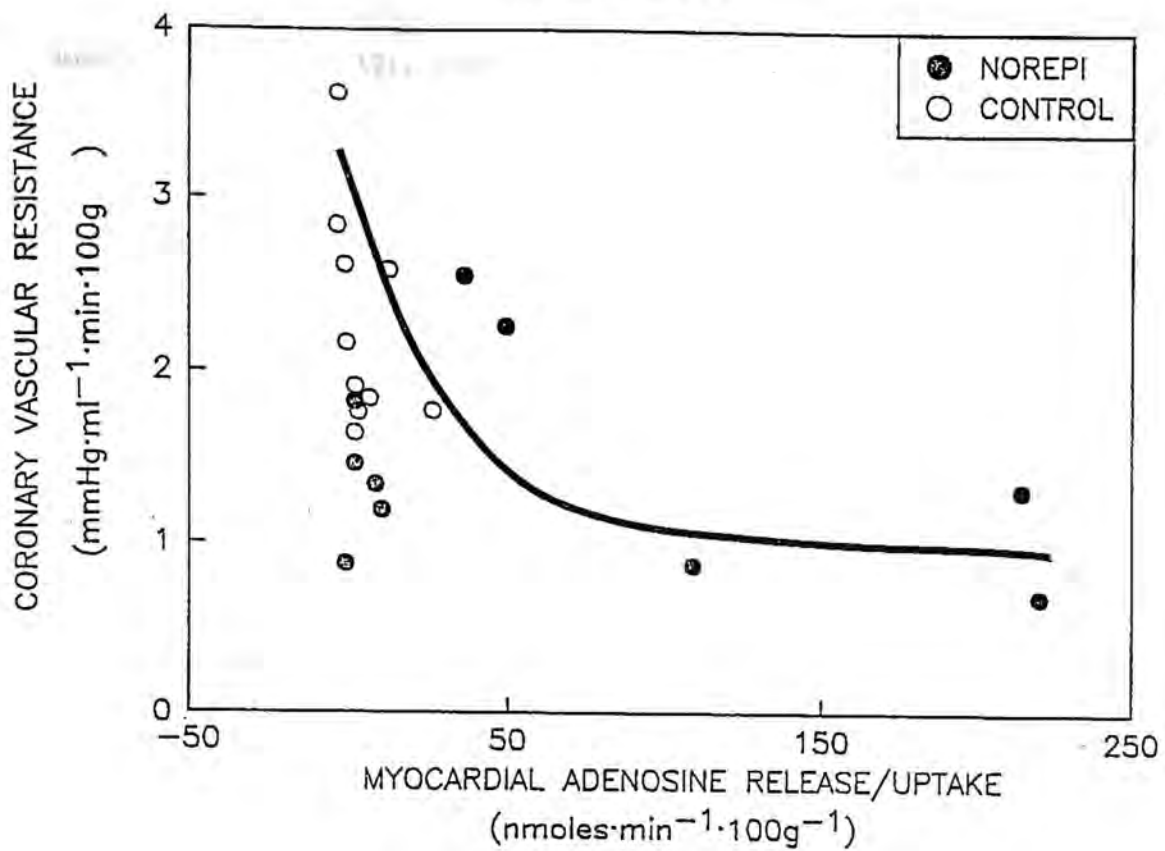
DIABETIC DOGS
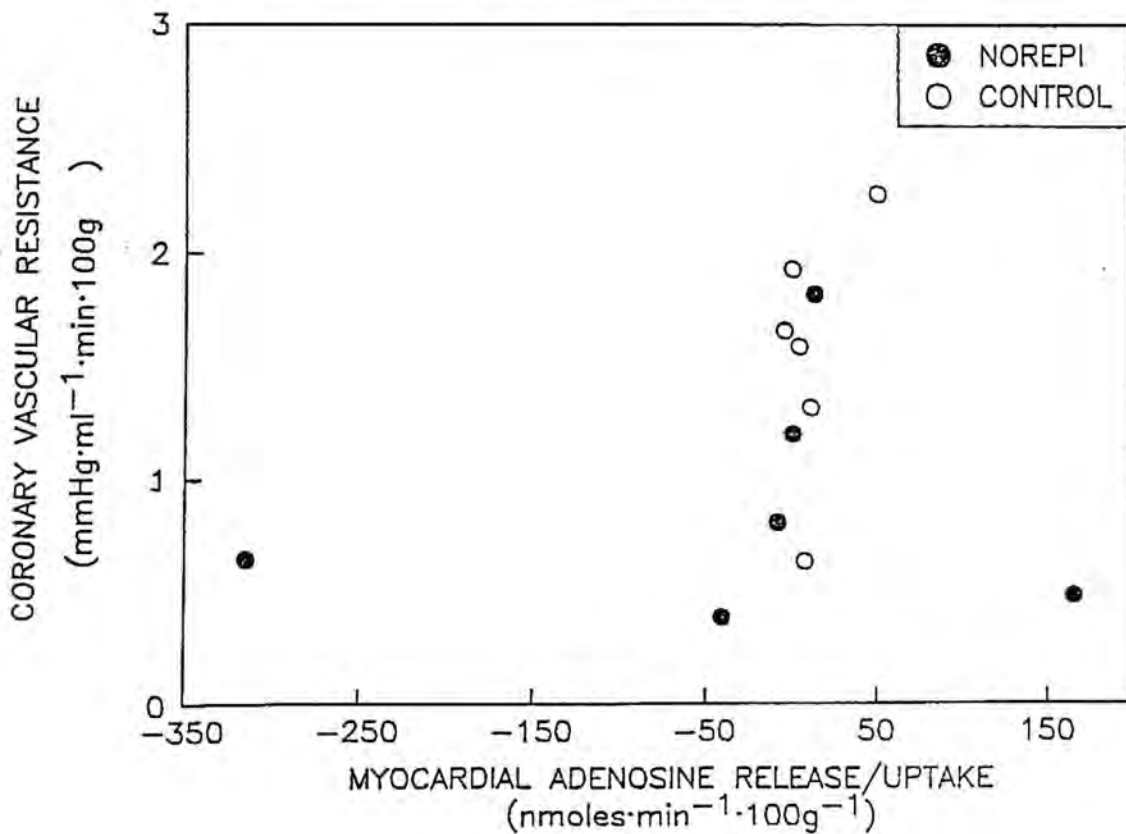
administration (n = 10), and prazosin control (PC) and prazosin

norepinephrine (PNE) refer to basal and stimulated states, respectively,

after prazosin administration (n = 7). $Alpha_1$ - blockade did not have a

significant effect on basal MABP, HR, or CO. However, these parameters

did not increase significantly with sympathetic stimulation after

prazosin as they did before prazosin, although there were no differences

between NNE and PNE MABP, HR, or CO. TPR remained constant after the

intracoronary injection of prazosin, indicating that the major effects of

the drug were limited to the myocardium. Basal LVP was decreased with

$alpha_1$ - blockade but LVP increased significantly (1.9 - fold) with

norepinephrine - stimulation as it did before $alpha_1$ - blockade (1.7 -

fold). Basal dP/dt and PRP (Fig. 24a) were unchanged after prazosin and

both increased significantly with sympathetic stimulation after prazosin.

There were no differences in LVP, dP/dt, or PRP between NNE and PNE

treatments.

Alpha$_1$ - blockade did not have a significant effect on CBF in

either basal or norepinephrine - stimulated states. CBF increased to the

same degree after $alpha_1$ - blockade (136%) as it did before $alpha_1$ -

blockade (140%). Neither basal nor norepinephrine - stimulated CVR were

significantly affected by $alpha_1$ - blockade, and CVR decreased

significantly with sympathetic stimulation after prazosin as it did

before prazosin (Fig. 24b).

2. Diabetic Dogs

Table 6B shows the effects of $alpha_1$ - adrenergic blockade on

hemodynamic parameters during control and norepinephrine - stimulated

TABLE 6A

HEMODYNAMIC DATA: NORMAL DOGS

$ALPHA_1$ - ADRENERGIC BLOCKADE STUDY

	NC (n = 10)	NNE (n = 10)	PC (n = 7)	PNE (n = 7)
MABP (mmHg)	114.9 ± 4.7	155.0 ± 7.8*	92.1 ± 3.3	128.8 ± 11.1
HR (bpm)	136.7 ± 7.3	175.1 ± 8.98*	158.3 ± 12.1	186.5 ± 8.7
TPR (mmHg· min·1^{-1})	51.2 ± 8.3	47.1 ± 4.1	39.8 ± 6.1	44.2 ± 2.3
CO (1/min)	2.52 ± 0.15	3.69 ± 0.43*	2.84 ± 0.54	3.14 ± 0.3
LVP (mmHg)	132 ± 6.3	225 ± 8.4*	106 ± 2.3†	207 ± 9.2*
dP/dt (mmHg/sec^2)	2775 ± 225	7750 ± 754*	2417 ± 396	8375 ± 482*
PRP (mmHg·bpm)	18148 ± 1419	39567 ± 2563*	17565 ± 1021	39343 ± 2665*
CBF (ml·min· $100g^{-1}$)	53.4 ± 4.3	128.2 ± 20.4*	52.2 ± 8.5	123.5 ± 19.1*
CVR (mmHg·ml^{-1}· min·100g)	2.27 ± 0.20	1.44 ± 0.19*	2.13 ± 0.38	1.08 ± 0.10*

values expressed as mean ± SEM
* $p < 0.05$ (compared to its own control)
† $p < 0.05$ (compared to same treatment before alpha - blockade)
NC = Normal Control   PC = Prazosin Control
NNE = Normal w/Norepinephrine   PNE = Prazosin w/Norepinephrine

Figure 24.   Effects of alpha$_1$ - adrenergic blockade on cardiac performance (a) and coronary vascular resistance (b) in normal dogs during basal and norepinephrine - stimulated conditions. Values are expressed as mean ± SEM for normal dogs (n = 6) in the control state (NC), with norepinephrine infusion (NNE), with prazosin in the control state (PC) and with prazosin and norepinephrine infusion (PNE).   *Denotes significant difference (p < 0.05) compared to its own control.

NORMAL DOGS
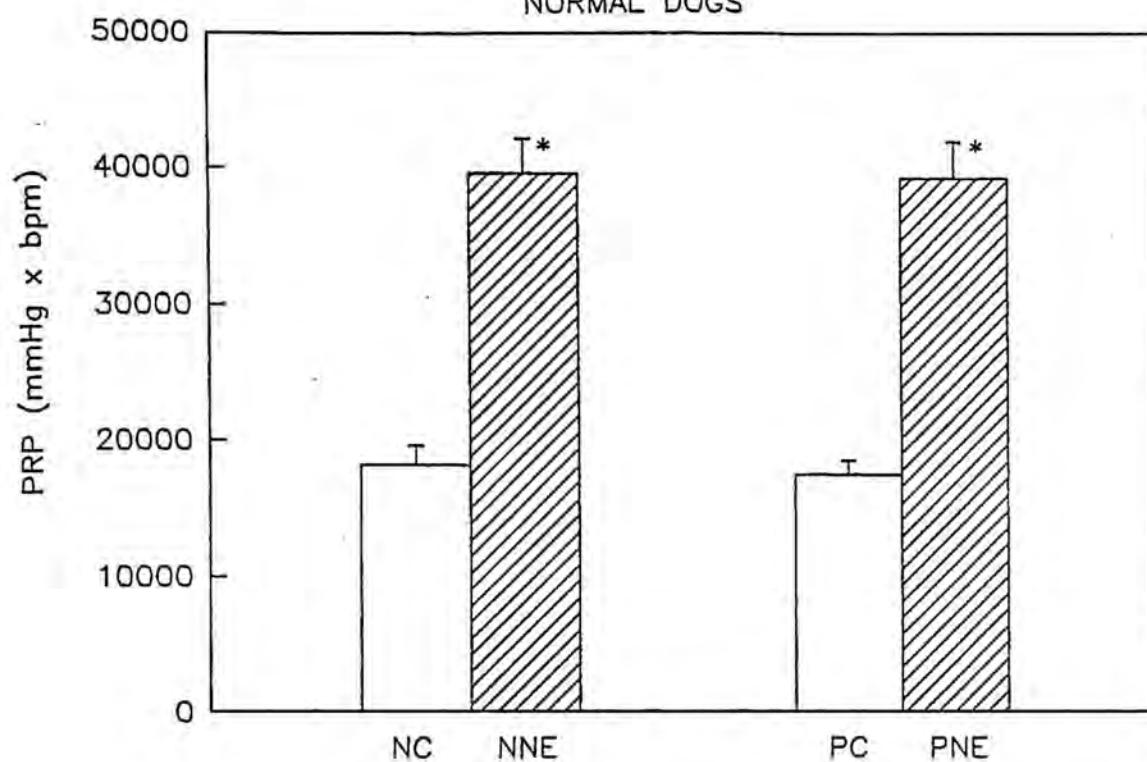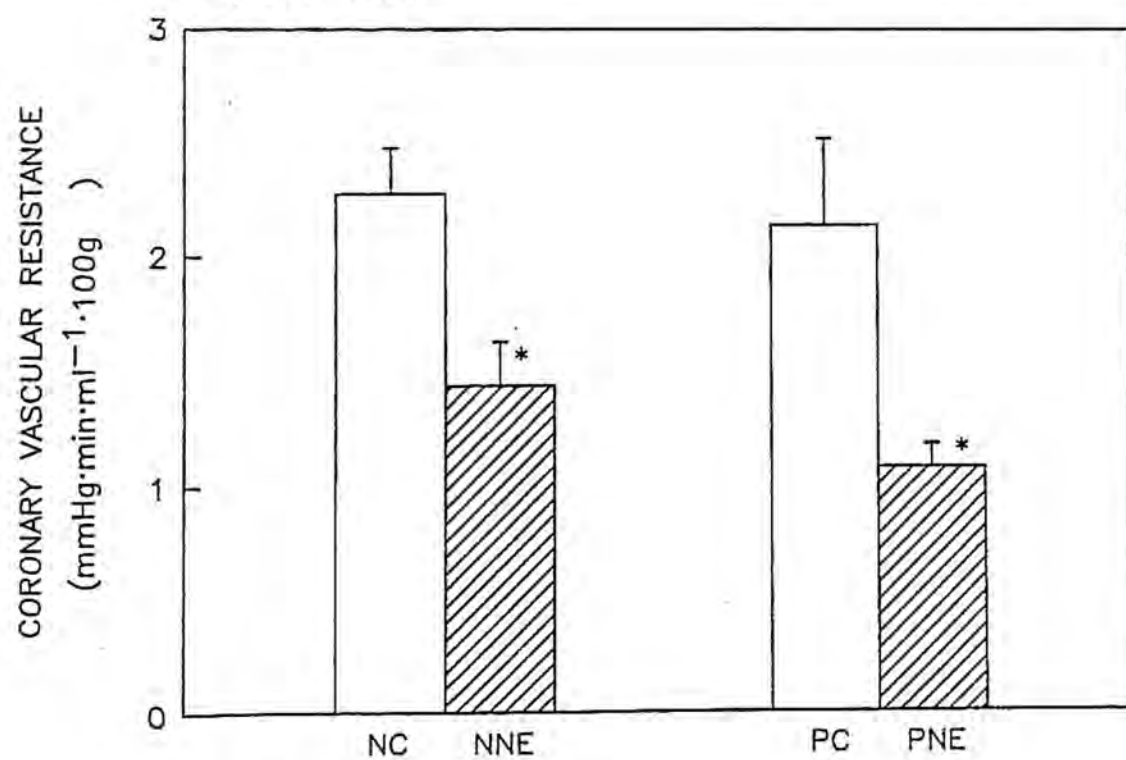
* † p < 0.05

* p < 0.05

conditions for diabetic dogs. For this table and the following diabetic dog tables, diabetic control (DC) and diabetic norepinephrine (DNE) represent basal and stimulated conditions, respectively, before intracoronary prazosin administration (n = 6), and diabetic prazosin control (DPC) and diabetic prazosin norepinephrine (DPNE) represent basal and stimulated conditions, respectively, after prazosin administration (n = 5). MABP was decreased significantly during $alpha_1$ - blockade in both basal and norepinephrine - stimulated states, and there was no increase in MABP with sympathetic stimulation during $alpha_1$ - blockade, as there were before $alpha_1$ - blockade. Prazosin had no effect on HR in either DPC or DPNE treatments, and HR increased significantly with sympathetic stimulation both before and after prazosin. As in the normal dogs, TPR remained constant through all treatments, indicating localization of prazosin in the myocardial region. There were also no significant changes in CO with any of the treatments in the diabetic dogs. Basal and stimulated dP/dt were unaffected by $alpha_1$ - blockade, and dP/dt increased significantly with sympathetic stimulation both before and after $alpha_1$ - blockade. Basal LVP and PRP (Fig. 25a) were decreased in the DPC compared to the DC treatments, but both parameters increased significantly in DNE and DPNE groups. In one of the diabetic dogs, LVP increased only 10mmHg before prazosin administration due to the presence of arrhythmias. In this dog, however, LVP was increased 63mmHg after prazosin, and there were no arrhythmias present.

Prazosin administration had no effect on basal CBF, but CBF did not increase significantly with norepinephrine after prazosin as it did before prazosin, although there were no differences between DNE and PNE

TABLE 6B

HEMODYNAMIC DATA: DIABETIC DOGS

ALPHA$_1$ - ADRENERGIC BLOCKADE STUDY

	DC (n = 6)	DNE (n = 6)	DPC (n = 5)	DPNE (n = 5)
MABP (mmHg)	135.3 ± 3.48	194.5 ± 12.7*	91.4 ± 5.36†	123.0 ± 19.5†
HR (bpm)	170.5 ± 11.2	211.7 ± 7.2*	148.2 ± 12.3	199.8 ± 15.1*
TPR (mmHg· min·1^{-1})	46.5 ± 2.24	77.04 ± 16.7	43.8 ± 7.23	53.6 ± 9.1
CO (1/min)	3.11 ± 0.27	4.21 ± 1.5	2.49 ± 0.39	2.51 ± 0.29
LVP (mmHg)	143.0 ± 2.8	229.5 ± 16.5*	104.6 ± 2.1†	217.0 ± 15.9*
dP/dt (mmHg/sec^2)	3245 ± 421	8500 ± 1016*	2650 ± 235	6600 ± 743*
PRP (mmHg·bpm)	23021 ± 1683	48303 ± 3207*	15502 ± 1347†	43331 ± 4454*
CBF (ml·min^{-1}· 100g^{-1})	103.6 ± 24.5	295.1 ± 77.7*	89.2 ± 19.4	226.7 ± 46.8
CVR (mmHg·min^{-1}· ml·100g)	1.56 ± 0.23	0.886 ± 0.22*	1.35 ± 0.26	0.621 ± 0.11*

values expressed as mean ± SEM
* $p < 0.05$ (compared to its own control)
† $p < 0.05$ (compared to same treatment before alpha$_1$ - blockade)
DC = Diabetic Control   DPC = Diabetic Prazosin Control
DNE = Diabetic w/Norepi.   DPNE = Diabetic Prazosin + Norepi.

Figure 25. Effects of alpha$_1$ - adrenergic blockade on cardiac performance (a) and coronary vascular resistance (b) in diabetic dogs during basal and norepinephrine - stimulated conditions. Values are expressed as mean $\pm$ SEM for diabetic dogs in the control state (DC), with norepinephrine infusion (DNE), with prazosin in the control state (PDC), and with prazosin and norepinephrine infusion (PDNE) *Denotes significant difference compared to its own control.

DIABETIC DOGS

PRP (mmHg × bpm)

* † p < 0.05

DIABETIC DOGS

CORONARY VASCULAR RESISTANCE (mmHg·min·ml^{-1}·100g)
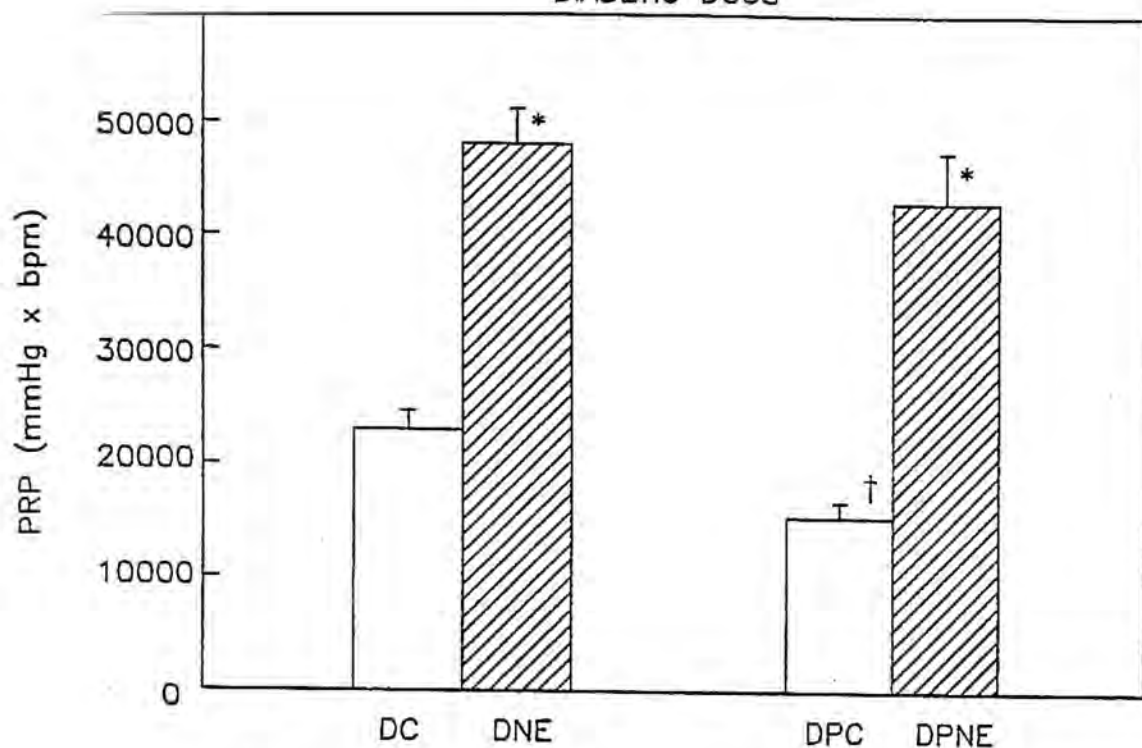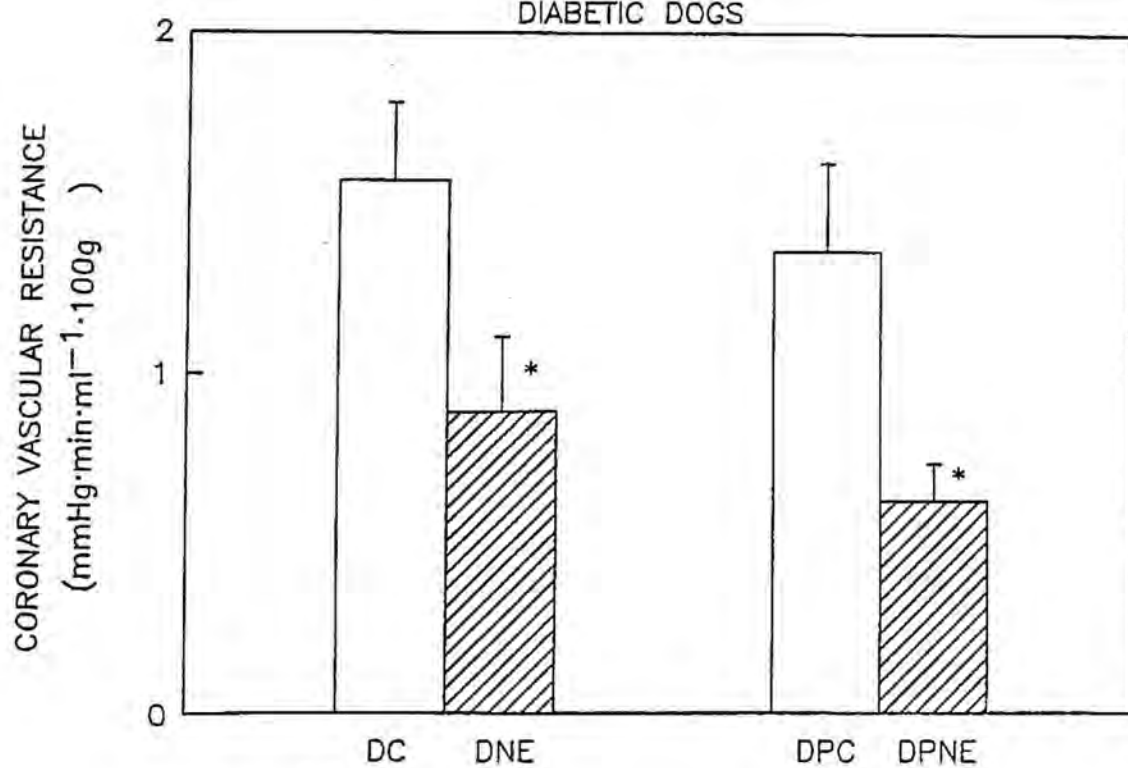
* p < 0.05

CBF measurements. Basal and norepinephrine - stimulated CVR were also unaffected by $alpha_1$ - blockade, and CVR decreased significantly with sympathetic stimulation both before and after prazosin (Fig. 25b).

b. Blood Gas Data

1. Normal dogs

Table 7A illustrates the effects of $alpha_1$ - adrenergic blockade on blood gas parameters before and during sympathetic stimulation in normal dogs. There were no differences in arterial blood PO_2, PCO_2, (O_2), pH, coronary sinus pH, or oxygen extraction between any of the four groups. Coronary sinus (O_2) increased with norepinephrine - stimulation before $alpha_1$ - blockade, but stayed constant during $alpha_1$ - blockade. Basal MVO_2 was unaffected by $alpha_1$ - blockade, and MVO_2 increased with sympathetic stimulation after prazosin as it did before prazosin.

2. Diabetic dogs

The effects of $alpha_1$ - blockade on blood gas parameters during control and norepinephrine - stimulated conditions in diabetic dogs are shown in Table 7B. There were no differences in arterial PCO_2, (O_2), coronary sinus pH, or arterial - coronary sinus O_2 between any of the treatments groups. Arterial PO_2 decreased with sympathetic stimulation during $alpha_1$ - adrenergic blockade, only, although arterial PO_2 did not differ between DNE and DPNE groups. Arterial pH decreased with norepinephrine administration before prazosin, but not after prazosin. However, there were no differences in arterial pH values between DC and DPC, or DNE and DPNE groups. Coronary sinus (O_2) did not increase with

TABLE 7A

BLOOD GAS DATA: NORMAL DOGS

$ALPHA_1$ - ADRENERGIC BLOCKADE STUDY

	NC (n = 10)	NNE (n = 10)	PC (n = 7)	PNE (n = 7)
Arterial PO_2 (mmHg)	83.6 ± 4.79	66.3 ± 2.28	74.3 ± 3.4	94.6 ± 29.7
Arterial PCO_2 (mmHg)	27.7 ± 1.4	30.7 ± 1.5	28.6 ± 2.1	32.9 ± 3.0
Arterial pH	7.434 ± 0.01	7.497 ± 0.02	7.412 ± 0.03	7.350 ± 0.02
CS pH	7.392 ± 0.01	7.341 ± 0.02	7.367 ± 0.03	7.332 ± 0.11
Arterial (O_2) $(mlO_2/100ml)$	20.32 ± 0.65	22.58 ± 0.95	20.94 ± 0.58	21.2 ± 0.10
CS (O_2) $(mlO_2/100ml)$	3.84 ± 0.33	7.15 ± 0.76*	4.0 ± 0.38	5.4 ± 0.92
A - CS (O_2) $(mlO_2/100ml)$	16.48 ± 0.75	15.43 ± 1.1	16.9 ± 0.5	15.7 ± 0.67
MVO_2 $(mlO_2 \cdot min^{-1} \cdot 100g^{-1})$	8.48 ± 1.04	18.28 ± 3.57*	7.69 ± 0.92	17.1 ± 2.4*

values expressed as mean ± SEM
* $p < 0.05$ (compared to its own control)
† $p < 0.05$ (compared to same treatment before $alpha_1$ - blockade)
NC = Normal Control   PC = Prazosin Control
NNE = Normal w/Norepi.   PNE = Prazosin w/Norepi.

TABLE 7B

BLOOD GAS DATA: DIABETIC DOGS

$ALPHA_1$ - ADRENERGIC BLOCKADE STUDY

	DC (n = 6)	DNE (n = 6)	DPC (n = 5)	DPNE (n = 5)
Arterial PO_2 (mmHg)	83.3 ± 10.04	72.8 ± 5.13	77.2 ± 5.0	60.3 ± 4.6
Arterial PCO_2 (mmHg)	25.6 ± 0.88	28.1 ± 2.3	25.5 ± 0.59	31.8 ± 2.5
Arterial pH	7.428 ± 0.01	7.326 ± 0.03*	7.375 ± 0.03	7.296 ± 0.02
CS pH	7.383 ± 0.02	7.330 ± 0.07	7.340 ± 0.03	7.260 ± 0.02
Arterial (O_2) $(mlO_2/100ml)$	20.52 ± 0.87	24.18 ± 1.33	17.4 ± 0.6	18.5 ± 1.15
CS (O_2) $(mlO_2/100ml)$	3.48 ± 0.26	12.75 ± 1.89*	5.9 ± 1.1	7.64 ± 1.28
A - CS (O_2) $(mlO_2/100ml)$	17.03 ± 0.95	12.55 ± 1.3	11.5 ± 1.2	10.8 ± 0.86
MVO_2 $(mlO_2 \cdot min^{-1} \cdot 100g^{-1})$	17.29 ± 2.5	34.28 ± 11.4	10.1 ± 2.5	22.9 ± 3.9*

values expressed as mean ± SEM
* $p < 0.05$ (compared to its own control)
† $p < 0.05$ (compared to same treatment before $alpha_1$ - blockade)
DC = Diabetic Control   DPC = Diabetic Prazosin Control
DNE = Diabetic w/Norepi.   DPNE = Diabetic Prazosin w/Norepi.

norepinephrine during $alpha_1$ - blockade as it did before $alpha_1$ - blockade, although there were no differences between coronary sinus (O_2) values between DC and DPC, or DNE and DPNE treatments. There was a significant two - fold increase in MVO_2 in the diabetic dogs with norepinephrine infusion after prazosin administration, and there were no significant differences in MVO_2 in either basal or norepinephrine - stimulated conditions before or after prazosin.

    c. Metabolic Data

       1. Normal dogs

Table 8A illustrates metabolic parameters in normal dogs during basal and norepinephrine - stimulated conditions, both before and after prazosin administration. There were no differences in arterial or coronary sinus lactate or pyruvate concentrations, coronary sinus L/P ratios, arterial or coronary sinus glucose concentration or glucose uptake between any of the four groups.

       2. Diabetic dogs

Table 8B illustrates metabolic parameters for diabetic dogs during basal and norepinephrine - stimulated conditions, both before and after prazosin administration. There were no differences in arterial lactate or pyruvate concentrations, coronary sinus lactate or pyruvate concentrations, coronary sinus L/P ratios, arterial glucose concentrations, coronary sinus glucose concentrations, or glucose uptake between any of the groups.

    d. Adenosine Data

       1. Normal dogs

The effects of $alpha_1$ - blockade on resting and norepinephrine -

TABLE 8A

METABOLIC DATA: NORMAL DOGS

ALPHA$_1$ - ADRENERGIC BLOCKADE STUDY

	NC (n = 10)	NNE (n = 10)	PC (n = 7)	PNE (n = 7)
Arterial Lactate (mmol/l)	1.99 ± 0.5	1.61 ± 0.32	1.24 ± 0.48	1.47 ± 2.14
CS Lactate (mmol/l)	1.32 ± 0.28	1.28 ± 0.26	1.11 ± 0.58	1.20 ± 0.25
Arterial Pyruvate (mmol/l)	0.077 ± 0.01	0.057 ± 0.01	0.084 ± 0.02	0.071 ± 0.01
CS Pyruvate (mmol/l)	0.061 ± 0.01	0.064 ± 0.01	0.084 ± 0.01	0.092 ± 0.03
CS L/P (mmol/l)	17.9 ± 5.8	16.9 ± 1.0	12.6 ± 3.5	15.1 ± 3.1
Arterial Glucose (mg/100ml)	87 ± 2.6	103 ± 6.4	85 ± 4.2	114 ± 13.7
CS Glucose (mg/100ml)	78 ± 5.7	89 ± 4.4	81.2 ± 7.1	112.4 ± 13.7
Glucose uptake (g·min^{-1}·100ml^{-1})	0.54 ± 0.44	1.57 ± 0.61	0.45 ± 0.17	0.23 ± 1.02

values expressed as mean ± SEM
NC = Normal Control
NNE = Normal w/Norepi.
PC = Prazosin Control
PNE = Prazosin w/Norepi.

TABLE 8B

METABOLIC DATA: DIABETIC DOGS

ALPHA$_1$ - ADRENERGIC BLOCKADE STUDY

	DC (n = 6)	DNE (n = 6)	DPC (n = 5)	DPNE (n = 5)
Arterial Lactate (mmol/l)	0.91 ± 0.25	1.49 ± 0.43	1.39 ± 0.5	1.31 ± 0.3
CS Lactate (mmol/l)	0.74 ± 0.25	1.45 ± 0.43	1.37 ± 0.5	1.54 ± 0.32
Arterial Pyruvate (mmol/l)	0.048 ± 0.01	0.064 ± 0.01	0.067 ± 0.01	0.069 ± 0.01
CS Pyruvate (mmol/l)	0.068 ± .004	0.101 ± 0.01	0.100 ± 0.02	0.100 ± 0.02
CS L/P (mmol/l)	11.18 ± 3.57	14.4 ± 5.1	14.3 ± 5.1	15.1 ± 4.9
Arterial Glucose (mg/100ml)	259 ± 23.4	341 ± 21.3	251 ± 22.6	311 ± 34.4
CS Glucose (mg/100ml)	227 ± 36.0	302 ± 29.0	258 ± 27.3	324 ± 36.9
Glucose uptake ($g \cdot min^{-1} \cdot 100g^{-1}$)	4.2 ± 1.6	9.3 ± 3.9	-1.78 ± 1.9	-1.23 ± 0.83

values expressed as mean ± SEM
DC = Diabetic Control
DNE = Diabetic w/Norepi.

DPC = Diabetic Prazosin Control
DPNE = Diabetic Prazosin w/Norepi.

stimulated adenosine parameters in normal dogs are shown in Table 9A. Arterial adenosine concentration remained constant throughout all treatments. Coronary sinus adenosine increased significantly with sympathetic stimulation before $alpha_1$ - blockade, but did not increase with sympathetic stimulation during $alpha_1$ - blockade. Similarly, there were no changes in adenosine across the myocardium or adenosine release (Fig. 26a) with norepinephrine - infusion during $alpha_1$ - blockade. Therefore, with the same increase in cardiac work and the same decrease in CVR, myocardial adenosine release was attenuated with $alpha_1$ - adrenergic blockade.

2. Diabetic dogs

The effects of $alpha_1$ - adrenergic blockade on arterial and coronary sinus adenosine concentrations, coronary sinus minus arterial adenosine concentrations, and adenosine release during basal and norepinephrine - stimulated conditions are shown in Table 9B. As shown in Fig. 26b, there were no differences in myocardial adenosine release between DC, DNE, DPC, or DPNE treatment groups. Thus, in diabetic dogs, $alpha_1$ - adrenergic blockade did not significantly affect adenosine release or the extent to which the coronary circulation is able to increase flow with sympathetic stimulation.

To summarize the $alpha_1$ - adrenergic blockade study, adenosine release with norepinephrine - mediated increases in cardiac work was attenuated in normal dogs with similar changes in cardiac work and coronary vascular resistance. $Alpha_1$ - adrenergic blockade did not seem to have a marked effect on the capability of the coronary circulation to

TABLE 9A

ADENOSINE DATA: NORMAL DOGS

ALPHA$_1$ - ADRENERGIC BLOCKADE STUDY

	NC (n = 10)	NNE (n = 10)	PC (n = 6)	PNE (n = 6)
Arterial Adenosine (pmoles/ml)	143.0 ± 37.3	203.6 ± 59.8	226.3 ± 51.9	227.7 ± 70.4
CS Adenosine (pmoles/ml)	188.5 ± 47.6	696.9 ± 201.0*	208.3 ± 58.0	283.5 ± 75.4
CS - Arterial Adenosine (pmoles/ml)	44.2 ± 44.9	492.4 ± 199.8*	-3.7 ± 50.0	35.7 ± 15.0
Adenosine Release/uptake (nmoles·min^{-1}· 100g^{-1})	3.9 ± 2.6	65.0 ± 27.5*	2.6 ± 5.0	14.6 ± 10.7

values expressed as mean ± SEM
* $p < 0.05$ (compared to its own control)
NC = Normal Control   PC = Prazosin Control
NNE = Normal w/Norepi.   PNE = Prazosin w/Norepi.

TABLE 9B

ADENOSINE DATA: DIABETIC DOGS

ALPHA$_1$ - ADRENERGIC BLOCKADE STUDY

	DC (n = 6)	DNE (n = 6)	DPC (n = 5)	DPNE (n = 5)
Arterial Adenosine (pmoles/ml)	194.8 ± 50.4	516.8 ± 281.0	466.3 ± 355.0	347.8 ± 144.0
CS Adenosine (pmoles/ml)	471.3 ± 156.0	388.1 ± 101.0	353.8 ± 77.7	769.8 ± 356.0
CS - Arterial Adenosine (pmoles/ml)	276.5 ± 156.0	-213.8 ± 28.5	-109.8 ± 399.0	184.3 ± 14.8
Adenosine Release/uptake (nmoles·min^{-1}· 100g^{-1})	11.42 ± 8.3	-39.6 ± 65.0	-6.06 ± 34.5	76.4 ± 34.7

values expressed as mean ± SEM
DC = Diabetic Control   DPC = Diabetic Prazosin Control
DNE = Diabetic w/Norepi.   DPNE = Diabetic Prazosin w/Norepi.

Figure 26. Effects of alpha$_1$ - adrenergic blockade on myocardial adenosine release/uptake in normal (a, n = 6) and diabetic (b, n = 5) dogs during basal and norepinephrine - stimulated conditions. Values are expressed as mean ± SEM for normal dogs in the control state (NC), with norepinephrine infusion (NNE), with prazosin in the control state (PC), and with prazosin and norepinephrine infusion (PNE), and for diabetic dogs in the control state (DC), with norepinephrine infusion (DNE), with prazosin in the control state (DPC), and with prazosin and norepinephrine infusion (DPNE). *Denotes significant (p < 0.05) from its own control.

NORMAL DOGS

* p < 0.05
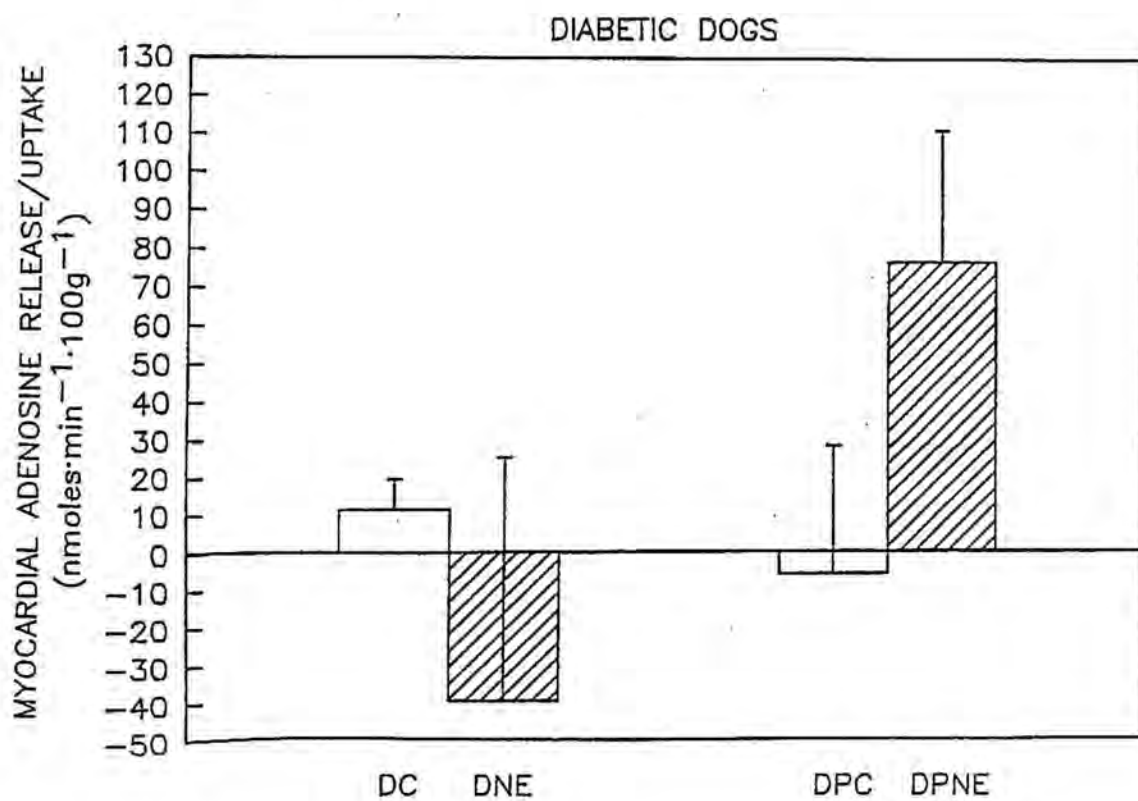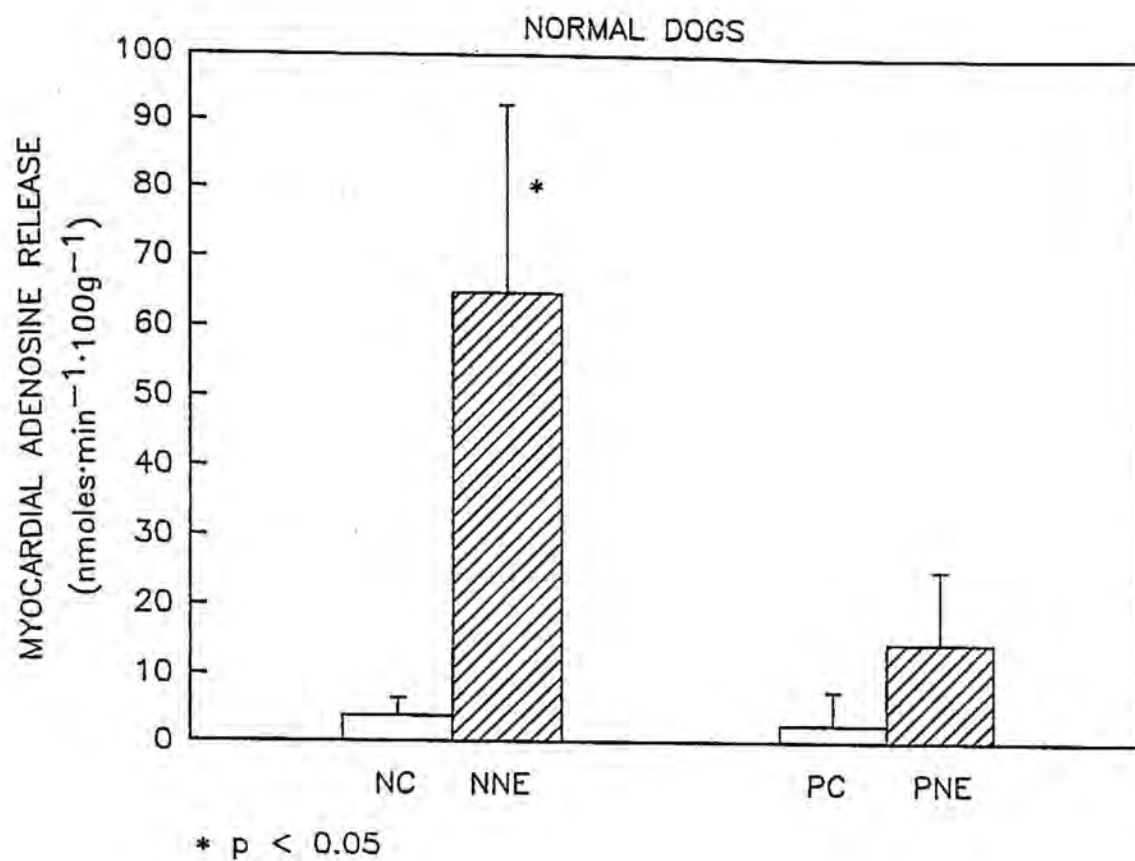
DIABETIC DOGS

vasodilate with sympathetic stimulation in either normal or diabetic dogs.

3. Acute Insulin Administration Study

a. Hemodynamic Data

1. Normal Dogs

The effects of accute insulin administration on resting and norepinephrine – stimulated hemodynamic parameters in five dogs are listed in table 10A. In this table and table 11A, NC, NIC, and NINE refer to control, insulin control, and insulin plus norepinephrine treatments in the normal dogs. Insulin alone had no significant effects on HR, TPR, CO, dP/dt, PRP, CBF, or CVR, although CVR decreased slightly in each dog after insulin. Mean arterial blood pressure and LVP were decreased in the resting state with insulin administration.

Sympathetic stimulation with insulin caused a 2.3 – fold increase in LVP, which was accompanied by a 3.1 – fold increase in CBF and a 0.5 – fold decrease in CVR. There were no significant differences in HR, TPR, CO, dP/dt, or PRP between NIC and NINE treatment groups.

2. Diabetic Dogs

The effects of acute insulin replacement on hemodynamic data at rest and during sympathetic stimulation for diabetic dogs are presented in Table 10B. Since only three dogs were used in this portion of the study, no statistical comparisons were made between group means. The individual values for each dog are listed for diabetic control (DC), diabetic insulin control (DIC), and diabetic insulin and norepinephrine – stimulated (DINE) conditions. Resting MABP decreased after insulin replacement in all three dogs, and MABP increased in all but one dog with norepinephrine and insulin. Heart rate decreased with insulin in two

TABLE 10A

HEMODYNAMIC DATA: NORMAL DOGS

ACUTE INSULIN ADMINISTRATION STUDY

	NC (n = 5)	NIC (n = 5)	NINE (n = 5)
MABP (mmHg)	124.0 ± 8.1	85.0 ± 1.6†	124.6 ± 14.7*
HR (bpm)	156.3 ± 8.5	158.0 ± 6.2	189.0 ± 12.5
TPR (mmHg·min· ml^{-1})	84.8 ± 29.5	47.3 ± 10.8	41.8 ± 6.9
CO ($l·min^{-1}$)	2.14 ± 0.50	2.34 ± 0.51	3.60 ± 0.81
LVP (mmHg)	142.5 ± 6.9	109.0 ± 4.6†	255.0 ± 20.0*
dP/dt ($mmHg/sec^2$)	1875 ± 139	2094 ± 328	9500 ± 3541
PRP (mmHg·bpm)	21440 ± 1931	17154 ± 606	47805 ± 4268
CBF ($ml·min^{-1}·$ $100g^{-1}$)	42.5 ± 7.9	42.2 ± 5.1	130.3 ± 31.8*
CVR ($mmHg·ml^{-1}·$ $min·100g^{-1}$)	3.28 ± 0.48	2.17 ± 0.33	1.12 ± 0.19*

values expressed as mean ± SEM
* $p < 0.05$ (compared to IC)
† $p < 0.05$ (compared to NC)
NC = Normal Control
NIC = Normal Insulin Control
NINE = Normal Insulin w/Norepi

TABLE 10B

HEMODYNAMIC DATA: DIABETIC DOGS

ACUTE INSULIN ADMINISTRATION STUDY

		DC	DIC	DINE
MABP	Dog 1	130	100	140
(mmHg)	Dog 2	110	75	130
	Dog 3	110	105	100
mean ± SEM		115 ± 5.0	95 ± 6.8	123 ± 12.0
HR	Dog 1	139	131	187
(bpm)	Dog 2	170	150	178
	Dog 3	158	171	183
mean ± SEM		158 ± 6.9	160 ± 20.5	183 ± 4.5
TPR	Dog 1	52.8	33.9	44.4
(mmHg·min·	Dog 2	39.7	23.8	15.1
ml^{-1})	Dog 3	37.4	31.2	21.9
mean ± SEM		46.1 ± 4.4	41.0 ± 11.6	27.1 ± 8.8
CO	Dog 1	2.46	2.94	3.16
(1/min)	Dog 2	2.77	3.15	4.56
	Dog 3	2.94	3.36	4.56
mean ± SEM		2.55 ± 0.2	2.69 ± 0.5	5.44 ± 1.6
LVP	Dog 1	135	108	170
(mmHg)	Dog 2	125	115	250
	Dog 3	135	135	270
mean ± SEM		131 ± 2.4	118 ± 5.3	230 ± 30.5
dP/dt	Dog 1	3500	2250	15000
($mmHg/sec^2$)	Dog 2	3500	7500	11500
	Dog 3	1750	2353	6500
mean ± SEM		2937 ± 413	4333 ± 1609	9833 ± 3562
PRP	Dog 1	18765	14148	31790
(mmHgxbpm)	Dog 2	21250	17250	44500
	Dog 3	21330	23085	49410
mean ± SEM		20731 ± 659	19332 ± 3742	38145 ± 6355
CBF	Dog 1	54.9	42.8	101.5
($ml·min^{-1}$·	Dog 2	47.4	53.3	246.5
	Dog 3	48.7	65.1	76.2
mean ± SEM		64 ± 13.7	54 ± 4.5	141 ± 53.0
CVR	Dog 1	2.37	2.34	1.38
($mmHg·ml^{-1}$·	Dog 2	2.32	1.43	0.53
min·100g)	Dog 3	2.26	1.61	1.31
mean ± SEM		2.0 ± 0.32	1.81 ± 0.20	1.07 ± 0.27

DC = Diabetic Control
DIC = Diabetic Insulin Control
DINE = Diabetic Insulin w/Norepi.

dogs, and increased in one dog, and sympathetic stimulation caused increases in heart rates in all three dogs. There were decreases in basal TPR with insulin in all dogs. Norepinephrine caused TPR to decrease further in two of the dogs and to increase in one dog. Resting CO increased with insulin, and norepinephrine - stimulated CO increased with respect to insulin control CO in all three dogs. Insulin caused decreases in LVP in 2 dogs and there was no change in one dog, and LVP increased in all three dogs with norepinephrine. There were increases in dP/dt in two dogs and a decrease in dP/dt in one dog with insulin. DP/dt increased in each dog with sympathetic stimulation. Two dogs showed decreases and one dog showed an increase in basal PRP with insulin, and PRP increased with norepinephrine over the DIC PRP in each dog. Coronary blood flow increased in two dogs and decreased in one dog with insulin replacement. Sympathetic stimulation caused increases in CBF in all three dogs. There were decreases in CVR in each dog with insulin alone and there were further decreases with insulin and norepinephrine.

   b. Adenosine Data

     1. Normal dogs

     Table 11A shows plasma adenosine parameters for NC, NIC, and NINE treatment groups. Basal arterial and coronary sinus adenosine concentrations were not affected by the single dose of insulin. Subsequently, there were no changes in the differences between coronary sinus and arterial adenosine concentrations or adenosine release with insulin. As opposed to the previous study where norepinephrine - stimulation caused increases in coronary sinus and coronary sinus minus arterial adenosine concentrations and adenosine release, there were no increases in these parameters with sympathetic stimulation after insulin

TABLE 11A

ADENOSINE DATA: NORMAL DOGS

ACUTE INSULIN ADMINISTRATION STUDY

	NC (n = 5)	NIC (n = 5)	NINE (n = 5)
Arterial Adenosine (pmoles/ml)	1113.6 ± 196.4	1420.8 ± 681.0	1148.0 ± 359.1
CS Adenosine (pmoles/ml)	833.1 ± 197.9	844.5 ± 249.6	1168.6 ± 364.4
CS - Arterial Adenosine (pmoles/ml)	-337.2 ± 250.6	-477.4 ± 540.2	20.2 ± 141.9
Adenosine Release/uptake (nmoles·min^{-1}· 100g^{-1})	-23.7 ± 17.1	-23.6 ± 26.2	-5.2 ± 12.7

values expressed as mean ± SEM
NC = Normal Control
NIC = Normal Insulin Control
NINE = Normal Insulin w/Norepi.

TABLE 11B

ADENOSINE DATA: DIABETIC DOGS

ACUTE INSULIN ADMINISTRATION STUDY

		DC	DIC	DINE
Arterial	Dog 1	904	996	2681
Adenosine	Dog 2	2689	580	430
(pmoles/ml)	Dog 3	369	328	212
mean ± SEM		1321 ± 701	635 ± 195	1108 ± 789
CS Adenosine	Dog 1	6540	1275	2238
(pmoles/ml)	Dog 2	2199	181	898
	Dog 3	219	487	190
mean ± SEM		2986 ± 1867	648 ± 326	1109 ± 600
CS - Art.	Dog 1	5636	279	-443
Adenosine	Dog 2	-490	-399	468
(pmoles/ml)	Dog 3	-150	159	-22
mean ± SEM		1665 ± 2869	13 ± 209	296 ± 159
Adenosine	Dog 1	309.0	11.9	-45.0
Release/	Dog 2	-23.2	-21.3	115.4
uptake	Dog 3	-7.3	10.4	-1.7
(nmoles min^{-1} ·100g)				
mean ± SEM		92.8 ± 110	0.35 ± 10.8	22.9 ± 47.9

DC = Diabetic Control
DIC = Diabetic Insulin Control
DINE = Diabetic Insulin w/Norepi.

administration.

2. Diabetic dogs

Table 11B illustrates adenosine parameters for each of the three dogs in the DC, DIC, and DINE treatment groups. Resting arterial adenosine stayed relatively ocnstant in two dogs and decreased in one dog with insulin replacement. Coronary sinus adenosine decreased in two dogs and increased in one dog after insulin. The change in adenosine across the myocardium decreased with insulin in two dogs and increased in one dog. Insulin caused a decrease in resting adenosine release in two dogs and an increase in resting adenosine release in one dog.

When compared to insulin control, sympathetic stimulation with insulin administration resulted in a decrease in arterial adenosine concentration in two dogs and an increase in arterial adenosine concentration in one dog. Coronary sinus adenosine increased in two dogs with norepinephrine and insulin and decreased in one dog. The change in adenosine across the myocardium and adenosine release decreased in two dogs with norepinephrine - stimulation and increased in one dog.

c. Adenosine Reactivity and Reactive Hyperemia

1. Normal dogs

Figure 27a illustrates the coronary flow responses to four intracoronary doses of adenosine, before and after insulin administration, respectively. These data are expressed as a percent of maximal vasodilation to exogenous adenosine. Mean resting percent of maximal coronary flow and the coronary flow responses to the two lower doses of adenosine (1.8 and 3.7 x 10^{-8}moles) were significantly increased in normal dogs with insulin as compared to normal dogs without added insulin. Thus these data suggest that insulin enhanced the coronary

TABLE 12

REACTIVE HYPEREMIA DATA

ACUTE INSULIN ADMINISTRATION STUDY

	CONTROL RH (ml)	INSULIN RH (ml)	CONTROL RH/BASE	INSULIN RH/BASE
Normal Dogs				
Dog 1	10.3	17.8	0.43	0.55
Dog 2	3.14	1.8	0.16	0.22
Dog 3	2.5	2.46	0.28	0.22
Dog 4	1.24	1.35	0.10	0.13
Dog 5	13.5	13.0	0.9	0.87
mean ± SEM	6.14 ± 2.4	7.28 ± 3.4	0.37 ± 0.14	0.40 ± 0.14
Diabetic Dogs				
Dog 1	13.6	12.5	0.56	0.52
Dog 2	6.66	3.77	0.55	0.18
Dog 3	9.5	4.5	0.1	0.04
mean ± SEM	9.92 ± 2.0	6.92 ± 2.8	0.40 ± 0.40	0.25 ± 0.14

reactivity to intraluminally applied adenosine.

As shown in Table 12, there was no change in absolute reactive hyperemia flow following a 5 - second occlusion after insulin administration. There were also no differences in the ratios of reactive hyperemia flows to the base flows at the time of occlusion between control and insulin treatments.

2. Diabetic dogs

Figure 27b illustrates the mean flow responses, expressed as a percentage of maximal flow response, to four doses of infused adenosine. Unlike the normal dogs, there was no apparent difference between responses to intraluminal adenosine before and after insulin replacement. In addition, the reactive hyperemia responses to 5 - second occlusions decreased in two out of the three diabetic dogs after the insulin injection.

d. Glucose Data

1. Normal dogs

Fasting arterial plasma glucose concentrations before insulin administration ranged from 88 - 102 mg/100 ml plasma, with a mean value of 94.3 ± 4.1 mg/100 ml plasma. After insulin, plasma glucose concentrations ranged from 29 - 51 mg/100 ml plasma, with a mean value of 40.0 ± 6.3 mg/100 ml plasma in the basal state, and ranged from 43 - 67 with a mean of 60.3 ± 5.1 after norepinephrine - stimulation.

2. Diabetic dogs

Fasting arterial plasma glucose concentrations ranged from 185 - 220 mg/100ml plasma in the control state with a mean of 206.3 ± 10.8, and ranged from 119 - 177 mg/100 ml plasma after insulin with a mean of 153 ± 17.5. Norepinephrine — stimulated arterial glucose concentrations ranged

Figure 27. Effects of insulin on vasodilatory responses to intracoronary
adenosine in normal (a, n = 5) and diabetic (b, n = 3) dogs. Values are
expressed as mean ± SEM for normal and diabetic dogs without insulin (squares,
solid lines) and for normal and diabetic dogs with insulin (triangles, dotted
lines). *Denotes significant difference (p < 0.05) compared to its own
control.

NORMAL DOGS
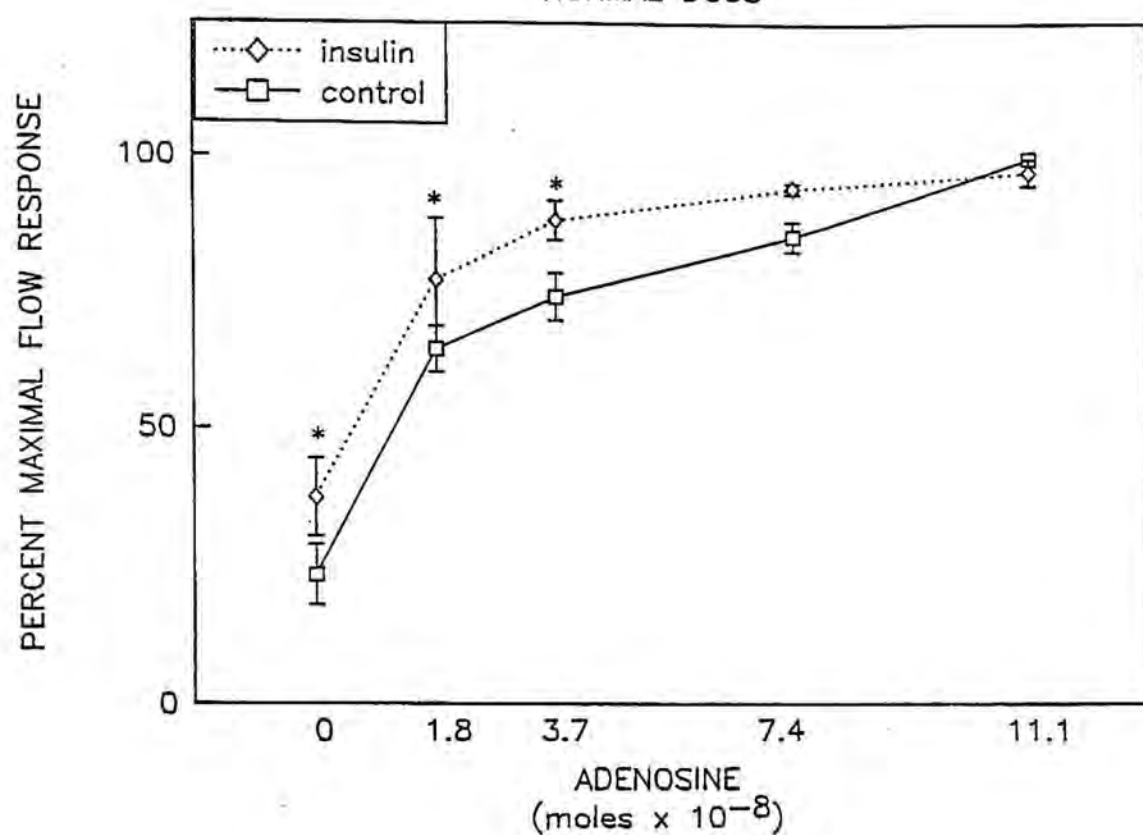
DIABETIC DOGS
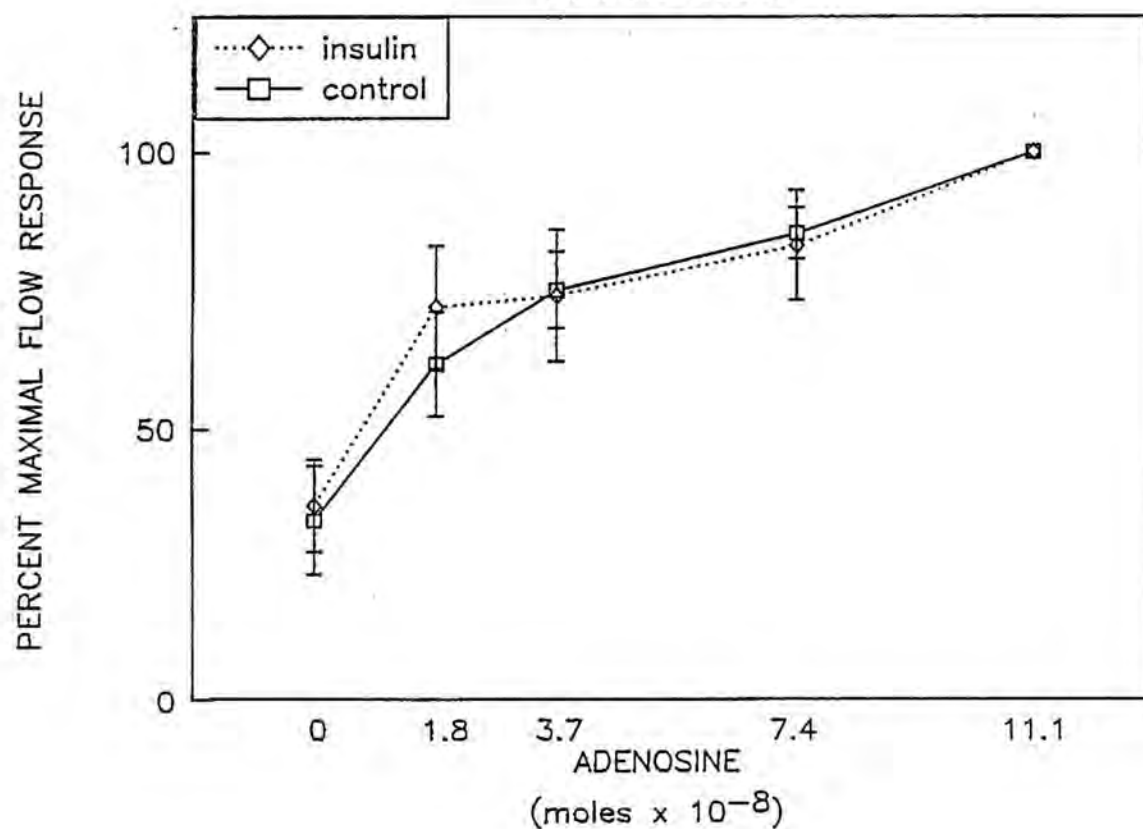
from 104 - 180 mg/100 ml plasma with a mean of 144 ± 15.8. Although blood glucose concentrations were lowered with insulin, the doses of insulin used in this study did not normalize blood glucose levels in the diabetics.

To summarize the insulin - replacement study, norepinephrine - mediated increases in cardiac work caused decreases in CVR in both normal and diabetic dogs after insulin administration. These changes in resistance were not accompanied by rises in adenosine release in either group. Normal dogs were more sensitive to intracoronary adenosine after insulin, and diabetic dogs did not show enhanced sensitivity to adenosine with the insulin dose used in this study. Reactive hyperemia was not enhanced by insulin administration in either group.

VI DISCUSSION

These studies were conducted in order to address the hypothesis
that the relationship between myocardial adenosine production and
coronary vascular resistance is altered in diabetes mellitus. It was
demonstrated that in the eight - week streptozotocin - induced diabetic
rat, a model that exhibited decreases in ventricular performance and
coronary blood flow, myocardial adenosine content was elevated and did
not correlate with coronary vascular resistance. Furthermore, in this
model of diabetes, the correlation between myocardial adenosine content
and coronary vascular resistance was restored with exercise conditioning.
In the 10 - week alloxan/streptozotocin - induced diabetic dog, a model
that did not exhibit reduced ventricular performance, it was shown that
myocardial adenosine release also did not correlate with coronary
vascular resistance, nor did adenosine release increase with
norepinephrine - stimulated increases in cardiac work as it did in normal
animals. There was no evidence of competition between neurally mediated
vasoconstriction and metabolic vasodilation with norepinephrine infusion
in the diabetic dogs whereas there did appear to be such competition in
the normal dogs. Finally, hyperemic responses to infused adenosine and
brief coronary occlusions were shown to be normal in the diabetic dogs,
but acute insulin administration, which increased adenosine sensitivity
in normal dogs, had no effect in the diabetic dogs. These findings
demonstrated that the relationship between adenosine production and
coronary vascular resistance is altered in diabetes.

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Several criteria for the metabolic regulation of coronary blood flow are fulfilled by the nucleoside, adenosine, and implicate adenosine as a possible metabolic vasodilator in normal hearts. However, studies examining the role of adenosine in the regulation of coronary blood flow in pathological states are lacking, and diabetes, with its associated cardiac complications, serves as an appropriate pathological model.

A. Basal Myocardial Adenosine Content and Coronary Vascular Resistance

In the first study, myocardial adenosine content was measured in the streptozotocin - induced diabetic rat to study the relationship between adenosine production and coronary vascular resistance in the basal state. Although adenosine release cannot be measured in the in - vivo rat heart, changes in tissue content of adenosine have been shown to correlate with coronary sinus and coronary lymph adenosine concentrations and adenosine release in the dog (McKenzie, et al., 1984). In addition, myocardial adenosine content has been shown to increase with decreases in coronary vascular resistance at rest and with isoproteranol - mediated increases in cardiac work in the open – chest rat model used in this study (McKenzie, 1983). The content of myocardial adenosine in the normal rats was within the range of adenosine contents measured in other open - chest rat preparations (Berne et al., 1971; McKenzie, 1983). However, resting myocardial adenosine content was significantly increased in the diabetic rat (Fig. 8). Increases in cardiac adenosine are usually caused by severe hypoxia or increases in cardiac work (Berne, 1980). The diabetic rats were not hypoxic, according to blood gas measurements. In addition, compared to normal rats, they exhibited decreased left ventricular pressure development, dP/dt, and pressure - rate - product,

similar to changes seen in isolated heart preparations from diabetic animals (Penparkgul et al., 1981). These decreases in ventricular function were also associated with a decrease in basal coronary blood flow. Whether the impaired ventricular function is a result of limited coronary blood flow, or the decreased coronary blood flow is appropriate for the decrease in ventricular work, is difficult to speculate without information on myocardial oxygen extraction and consumption in the diabetic rats.

Stimulation of adenosine production during increased cardiac work is assumed to be linked to increased oxygen consumption (Mohrman and Feigl, 1978). It is possible that oxygen consumption was elevated in the diabetic rats due to biochemical changes in mitochondrial or contractile ATPase function, causing an increase in the ratio of oxygen demand to unit work. In the isolated diabetic rabbit heart, for example, there was an uncoupling between myocardial oxygen consumption and ventricular work, with oxygen consumption exceeding normal levels at given ventricular filling pressures (Fields, 1984). The inability to measure myocardial oxygen consumption in the open chest model prevented us from assessing the relationship of myocardial oxygen consumption to ventricular performance in this preparation.

The absence of a decrease in coronary vascular resistance with the increase in myocardial adenosine raises several questions. It is possible that the intracellular myocardial adenosine storage pool is increased, and that the elevated adenosine content does not reflect interstitial adenosine. Endothelial degradation of adenosine may also be impaired in the diabetic animals, since the endothelium undergoes

degenerative processes in diabetes (McMillan, 1975). Assuming that the increased adenosine content represents an interstitial vasoactive pool, it is possible that the elevation in adenosine production was in response to an increase in alpha - adrenergic vasoconstrictor tone in the diabetic animals. If the increased constrictor tone was limiting oxygen supply, then this would cause more adenosine to be produced and released in order to maintain the same coronary blood flow. If the adrenergic activity varied between diabetic rats, then the lack of correlation between adenosine and coronary resistance observed when each rat was plotted individually would not necessarily negate a role for adenosine in coronary blood flow regulation in diabetes. In other words, each rat would have its own degree of neural constriction to overcome metabolically. Otherwise, these data indicate that adenosine does not regulate coronary vascular resistance in the diabetic rat.

Another possible explanation for the increase in adenosine production without a change in resistance is a decrease in adenosine receptor sensitivity. Vascular responsiveness to infused adenosine has been shown to be diminished in diabetic heart preparations where virtually all insulin has been removed from the system (Downing and Lee, 1985, Liang and Belloni, 1986). The response to adenosine was restored upon insulin replacement (Downing, 1985), suggesting that there may be a cooperative relationship between insulin and adenosine action.

B. Basal Myocardial Adenosine Content and Exercise Conditioning

Exercise conditioning, which affects the activity of the autonomic nervous system and insulin sensitivity in both normal and diabetic individuals, was employed to examine its effects on myocardial

adenosine content and coronary vascular resistance. In agreement with Barnard *et al.* (1980), both normal and diabetic rats demonstrated decreases in coronary blood flow and increases in coronary vascular resistance after a running program. As suggested by Knight and Stone (1983), these changes may be indicative of enhanced alpha - adrenergic activity. The increase in myocardial adenosine content with exercise training in normal animals (Fig. 7) was also observed in a study by McKenzie (1983), in which a swimming program was used to condition the rats. In that study, myocardial catecholamine content was also elevated, suggesting that there might have been increased adrenergic activity. In further support of a uniform enhancement of alpha - adrenergic tone is the rightward shift of the correlation curve between adenosine content and coronary resistance (Fig. 10), showing a greater degree of resistance for a given content of adenosine.

The shift in the adenosine vs coronary vascular resistance curve could also be interpreted to suggest a decrease in adenosine receptor sensitivity, especially since coronary blood flow was decreased in the exercised - trained rats. However, exercise - training is associated with an increase in insulin receptor sensitivity in most tissues (Koivisto *et al.*, 1979; Pederson *et al.*, 1980), and as mentioned previously, insulin is proposed to enhance adenosine receptor sensitivity (Downing, 1985). This is exemplified by the restoration of the correlation between adenosine and coronary vascular resistance in the exercise - conditioned diabetic rats. The improved correlation between adenosine and coronary vascular resistance in the diabetic rats after training is consistant with a role for adenosine mediation of coronary

blood flow in the diabetic rats after training.

Although the higher level of adenosine did not override the increased vascular resistance after training, as shown by a decrease in CBF, there was not a decrease in ventricular function after training Figures 6a and b). The lower coronary blood flow was probably adequate to meet the needs of the myocardium, since mitochondrial enzyme capacity has been shown to increase with exercise conditioning (Lamb, 1978), thus allowing a greater rate of oxygen extraction per unit work or a more favorable myocardial oxygen supply/demand balance (Barnard et al., 1980). Unfortunately, the in - vivo rat heart is too small to allow the measurement of coronary venous oxygen content, so oxygen extraction and consumption information could not be attained.

C. Basal and Norepinephrine Stimulated Adenosine Release and Coronary Vascular Resistance

The preliminary questions raised in the diabetic rat study concerning adenosine compartmentalization vs release, the relationship between adenosine production and cardiac metabolism, and changes in vasoconstrictor tone with diabetes made it necessary to study the potential role of adenosine in mediating coronary blood flow in an animal that could provide information on adenosine release and oxygen consumption. The 10 - week diabetic dogs differed from the 8 - week diabetic rats hemodynamically, but lent support to the presence of an alteration in metabolic regulation of coronary blood flow. Unlike the diabetic rats, the diabetic dogs did not show impaired ventricular function. In fact, due to a significant increase in heart rate, pressure - rate - product was elevated in the diabetic dogs. The increase in

cardiac performance was accompanied by increased coronary blood flow and decreased coronary vascular resistance. The conditions found in the alloxan/streptozotocin diabetic dog more closely resemble early stages of human diabetes, which are associated with states of hyperfunction and hyperperfusion of organs deleteriously affected several years after the onset of the disease symptoms (Theusen et al., 1986). In a study by Koltai et al. (1984), as in this study, there were no differences in ventricular pressure development or dP/dt in three - month alloxan - diabetic dogs compared to normal dogs. However, the dogs in Koltai's study showed no differences in resting heart rate or coronary blood flow. Like the increase in myocardial adenosine content in the diabetic rats, the diabetic dogs exhibited significant increases in coronary sinus adenosine in the basal state but adenosine release was not significantly elevated in the diabetic dogs. There was also an increase in basal myocardial oxgyen consumption in the diabetic dogs, but this was appropriate for the increase in cardiac performance.

D. Metabolic Blood Flow Regulation and Ventricular Function

Studying changes in hemodynamic and metabolic parameters with sympathetically - mediated changes in cardiac work unmasked differences in indices of coronary blood flow regulation between normal and diabetic dogs. In the normal dogs the increase in cardiac work caused increases in coronary sinus adenosine concentration, adenosine release and oxygen consumption, and a decrease in coronary vascular resistance, with no change in oxgyen extraction. The constant level of oxygen extraction with an increase in coronary blood flow is indicative of a metabolic vasodilation (Feigl, 1983), because the increased metabolic demand is

matched by an increase in coronary blood flow. Since the increased adenosine release correlated with increases in oxygen consumption and decreases in coronary vascular resistance, the possibility of an adenosine - mediated hyperemia is supported in the normal dogs. In the diabetic dogs, however, the increase in cardiac work produced by norepinephrine infusion did not cause an increase in coronary sinus adenosine concentration or adenosine release, although there were increases in coronary blood flow and decreases in coronary vascular resistance. In fact, the changes in coronary blood flow, coronary resistance, heart rate, arterial blood pressure, and pressure - rate - product were greater in the diabetic dogs than in the normal dogs. This observation was not made in Koltai's study, in which cardiac work was increased with stimulation of sympathetic cardiac nerves, instead of norepinephrine infusion. Sympathetic stimulation caused vasoconstriction in diabetic dogs, as opposed to vasodilation in normal dogs (1984).

The decrease in oxygen extraction and the large decrease in coronary vascular resistance in our diabetic dogs are indicative of either a non - metabolic vasodilation, or an increased response to another vasodilator such as K^+ or CO_2, released with norepinephrine - stimulated increases in cardiac work. Although arterial and coronary sinus K^+ concentrations were not measured in this study, K^+ release from the heart has not been shown to increase during catecholamine stimulation (Sarnoff et al., 1966). In addition, changes in coronary sinus CO_2 responses to norepinephrine were similar between normal and diabetic dogs.

It is possible that the hyperemic response to norepinephrine

stimulation in the diabetic dogs could have been mediated by neural factors. The anti - adrenergic effects of adenosine have been shown to be reduced in diabetic rat hearts (Liang and Belloni, 1986), which would enhance adrenergic responses to norepinephrine in diabetic animals. Although there is no direct evidence regarding the sensitivity of vascular beta - adrenergic receptors in diabetic dogs, increases in coronary blood flow are more closely correlated with indices of beta adrenergic activity in the diabetic dogs than in the normal dogs. If dP/dt, which is increased directly by beta - adrenergic stimulation, is used as an index of beta - activity, then beta - adrenergic sensitivity to norepinephrine seems to be enhanced in the diabetic dogs. Changes in oxygen consumption, coronary blood flow, and coronary vascular resistance correlate more closely with dP/dt in diabetic dogs than in normal dogs (Figures 16, 18, 19, 20). In addition, changes in myocardial oxygen consumption do not correlate with changes in pressure - rate - product in the diabetic dogs, as they do in the normal dogs (Figure 17). Pressure - rate - product is an index of cardiac performance that combines direct beta - mediated changes in heart rate and ventricular pressure with changes in ventricular pressure development related to increased preload, or venous return. Generally, increases in pressure have a greater effect on myocardial oxygen consumption than do increases in heart rate. Since cardiac compliance, or the ratio of changes in ventricular volume to changes in pressure, has been observed to be decreased in diabetic dogs (Regan et al., 1981), it is possible that the lack of correlation between oxygen consumption and pressure - rate - product are related to alterations in relative contributions of changes in ventricular pressure

development and heart rate. For example, if an increase in pressure - rate - product was mostly due to an increase in pressure development, than oxygen consumption would increase more than if the increase in pressure - rate - product was due to an increase in heart rate.

The absence of an increase in adenosine release, like the increase in oxygen extraction, with norepinephrine - stimulation, could also have been attributed to a beta adrenergic receptor - mediated vasodilation. If a beta - adrenergic mediated increase in coronary blood flow raises the ratio of oxygen supply to demand, then rephosphorylation of ADP to ATP would be favored over the breakdown of ADP to AMP. This increase in high - energy phosphates would cause the inhibition of 5'-nucleotidase activity thereby preventing an increase in adenosine release. A similar situation seemed to occur in the normal dogs with $alpha_1$ - adrenergic blockade. When $alpha_1$ - adrenergic constriction was removed in the normal dogs, thereby removing the competition between metabolic and neural vasoregulatory factors, adenosine release did not increase with norepinephrine stimulation. It is possible that the decrease in $alpha_1$ tone allowed $beta_2$ - adrenergic receptor stimulation to increase blood flow by neural means, and a smaller amount of adenosine was needed to enhance the vasodilation until the metabolic needs of the myocardium were met.

Although the attenuation of norepinephrine - stimulated adenosine release with $alpha_1$ - blockade in normal dogs supports the relationship between metabolic vasodilation and neural vasoconstriction during exercise, there is no indication of this relationship in the diabetic dogs. Similar to the normal dogs, there was no change in coronary sinus

adenosine concentration or adenosine release with prazosin and norepinephrine, but adenosine release in the diabetic dogs tended to be higher with prazosin and norepinephrine than with norepinephrine alone.

The altered relationships of myocardial adenosine release to coronary vascular resistance and cardiac work in the diabetic animals could stem from several sources, such as changes in factors controlling adenosine storage and release or changes in the efficacy of adenosine once released. In the final portion of this study we examined how acute insulin administration would affect adenosine release patterns and coronary resistance, and how the coronary circulation responds to infused adenosine and brief coronary occlusions. Unfortunately, only three diabetic dogs were able to complete this study, so it was difficult to quantify differences between the normal and diabetic dogs. However, we were able to make some important comparisons between in - vivo and in - vitro models and generate questions to be answered in future studies.

E. Insulin and Adenosine Sensitivity

Insulin administration caused decreases in basal left ventricular pressure development and arterial blood pressure in both normal and diabetic dogs, and decreases in coronary vascular resistance in every dog, although the mean decrease in resistance in the normal dogs was not significant. Insulin has coronary vasodilatory properties of its own, as shown by Downing and Lee in the neonatal lamb preparation, and like adenosine, insulin may protect the myocardium from catecholamine - induced injury (1979,1985). The action of insulin on vascular smooth muscle and cardiac muscle is due to a reduction in cAMP, which is proposed to reduce calcium entry into the cell, similar to adenosine's

proposed mechanism. In addition to it's direct effects, insulin may act in conjunction with adenosine to decrease vascular resistance, either at the receptor or post - receptor level. Evidence of this theory is based on the finding that the coronary dilator abilities of both insulin and adenosine have been shown to be reduced in the diabetic neonatal lamb, and restored by hypercapnea, which is known to enhance adenosine sensitivity (Downing and Lee, 1979; Merrill et al., 1978). In our study sympathetic stimulation caused a decrease in coronary vascular resistance without an increase in adenosine release in the normal dogs after insulin administration. It is possible, therefore, that adenosine release was attenuated in the normal dogs due to an increase in adenosine sensitivity, because incrased adenosine sensitivity would increase the feedback inhibition of hyperemia related increases in oxygen availability on adenosine prduction.

In the diabetic dogs, like the normal dogs, there was no pattern of an increase in adenosine release with norepinephrine stimulation. However, the diabetic dog who had the largest change in resistance (63%), had a 6.4 - fold increase in adenosine release, and the diabetic dog with the smallest changes in resistance (19%), showed a 1.4 - fold decrease in adenosine release. The differences between dogs were probably not due to differences in insulin sensitivity, since insulin produced 38 and 48% decreases in CVR in the first and second dogs mentioned above, respectively, in the basal state. Therefore it seems that changes in resistance were better related to changes in adenosine release after insulin replacement, which might have been due to improved adenosine sensitivity. Of course, more studies need to be done to confirm this

explanation.

Adenosine was injected into the coronary circulation in order to assess its sensitivity in diabetic dogs. Bolus injections of adenosine caused similar degrees of vasodilation in both normal and diabetic dogs. These results do not agree with findings of Downing and Lee(1985), Liang and Belloni (1986), or Koltai et al. (1984), but the experimental conditions of their preparations were different than ours. In all three studies, constant adenosine infusions were administered for 5 minutes instead of bolus injections, which we used to give instantaneous changes in coronary blood flow. It has been reported that tachyphylaxis occurs in skeletal muscle with prolonged adenosine infusions (Hester et al., 1982), and preliminary results obtained in our laboratory on isolated coronary artery strips from normal and diabetic dogs suggest that insulin may help prevent tachyphylaxis to repeated doses of adenosine. In these studies the vasodilatory responses to adenosine after several adenosine challenges in some of the preparations were enhanced after insulin administration. Although tachyphylaxis to adenosine has not been reported in the coronary circulation of normal dogs, it may play a role in adenosine sensitivity in diabetic dogs. Therefore the sustained response to a constant infusion of adenosine could be reduced in the preparations of Downing and Liang and Belloni due to decreased receptor sensitivity to adenosine secondary to insulin lack, while instantaneous responses are unchanged. In addition, while there is some circulating insulin in the diabetic dog, Liang and Belloni used an isolated system where there was no insulin present in the perfusate and Downing used a recirculating system that bypassed the splanchnic circulation, which may

have had lower insulin concentrations than our preparation. Whether adenosine is more physiologically involved in moment - to - moment adjustments of coronary vascular resistance, or more sustained changes in resistance is an area of dispute. In the conscious exercising dog, coronary sinus adenosine increases rapidly within the first two minutes, and then decreases with small pulsatile increases over the remainder of the exercise period (McKenzie et al, 1986). It has been suggested that adenosine is responsible for increases in coronary blood flow at the initiation of exercise, and that another vasodilator, such as potassium, may be involved with sustained increases in flow.

Insulin replacement produced an increased response to adenosine in the normal dogs but no change in response to adenosine in diabetic dogs. These findings also differ from those of Downing who showed no change in flow response to adenosine after insulin in normal lambs, and an increase in flow response after insulin in diabetic lambs (1985). Again, prolonged adenosine infusions were used, but the main difference was the dose of insulin administered. Downing gave an insulin dose of 10 units/kg, in contrast to 1.5 units/kg used in this study. A low dose was selected because it is within the range of doses used clinically to treat diabetic hyperglycemia. The amount of insulin injected into the normal dogs produced a 50% reduction in blood glucose concentration, and appeared to increase sensitivity to bolus injections of adenosine. The insulin given to the diabetic dogs only reduced glucose 25%, which was still above the normal range. No enhancement of adenosine sensitivity was observed with this insulin dose. In retrospect, blood glucose levels should have monitored before the second adenosine infusion to assure that

the insulin given was sufficient to produce normoglycemia. The doses of adenosine used in this study may also have been too high to pick up changes in sensitivity in the lower range. It is difficult to compare bolus concentrations with infused concentrations used in the various studies, and the other studies did not report the concentrations of adenosine used to produce maximal vasodilation. Nevertheless, our diabetic dogs were quite responsive to adenosine before as well as after insulin.

Similar to the adenosine infusion data, there were no obvious differences in reactive hyperemia responses to a 5 - second coronary occlusion between normal and diabetic dogs. In contrast, Koltai et al. reported decreased peak hyperemic responses and increased length of reactive hyperemia after a one - minute occlusion (1983). Again, our occlusion was for a much shorter period of time, and their results are more consistent with sustained adenosine response, whereas our occlusions represented quick changes in the release of metabolic dilators. Unlike our adenosine infusion study, insulin did not improve reactive hyperemia flow in normal or diabetic dogs. The anatomical differences between areas involved in the two hyperemic processes could be responsible for the discrepency in insulin's effects. It is possible that intraluminal and extraluminal adenosine receptors act differently with respect to insulin's influence on adenosine sensitivity. Reactive hyperemia, as opposed to hyperemia induced by adenosine infusion, is thought to act through adenosine receptors located on the extraluminal vascular smooth muscle cells. These receptors may not have been exposed to as much insulin as the intraluminal receptors. It is clear that these studies

need to be examined further to determine their significance with respect to insulin dosage and length of coronary occlusion.

F. Conclusions

This study has shown that the relationship between myocardial adenosine production and coronary vascular resistance is altered in the chemically - induced diabetic rat and dog. In both models, adenosine production, whether represented by tissue content or release, is unrelated to changes in cardiac work. Additionally, in the diabetic dog, adenosine release is not related to oxygen consumption either. We have shown that exercise training, which is associated with increased alpha - adrenergic coronary tone and insulin sensitivity, restores the relationship between myocardial adenosine content and coronary vascular resistance in the rat. Because insulin increased the sensitivity of the coronary vascular bed to infused adenosine and attenuated the increase in adenosine release with norepinephrine stimulation in the normal dogs, we feel that insulin may influence adenosine - mediated effects, either at the receptor or postreceptor level. If insulin increases receptor sensitivity to adenosine, possible mechanisms could involve an increase in extracellular H^+ ion concentration due to insulin's action on H^+ ion transport, or insulin could directly affect the conformation of the adenosine receptor. There could also be interaction between adenosine and insulin receptors. Post receptor mechanisms leading to an enhanced vasodilitory response could stem from the similar function of the two agents to decrease intracellular calcium fluxes. For example, insulin and adenosine alone decrease vascular resistance by reducing calcium influx. By acting simultaneously, there could either be an additive

effect or a potentiation of the vasodilation if intracellular interaction between second messengers responsible for the calcium antagonism occur Further studies are needed to elucidate the possible relationship between adenosine and insulin.

In conclusion, although exogenous adenosine did produce vasodilation in the diabetic dog, there was not a correlation between adenosine production and coronary vascular resistance in the diabetic dogs. Therefore, adenosine may not play a prominent role in coronary blood flow regulation in diabetes.

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